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STUDIES ON THE GLUTATHIONE STABILITY TEST AND ITS USE IN A CLINICAL LABORATORY*

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For some years this laboratory has been interested in the problem of the laboratory diagnosis of hemolytic disease. A battery of tests has been proposed to aid in the determination and definition of hemolytic mechanisms.[8] There are, however, hemolytic episodes which are not detected by these methods. Those associated with the so-called primaquine-sensitive erythrocytes are among them and it was toward the detection of these cells that our investigation turned.

The regular occurrence of hemolysis in certain individuals following the administration of primaquine led to the use of the term primaquine-sensitive erythrocytes. This sensitivity was thought to occur exclusively in the Negro race, but it is becoming increasingly evident that a relationship exists between primaquine-sensitive erythrocytes and diseases such as favism, napthalene poisoning in children, hemolysis in newborns due to vitamin K derivatives as well as hemolysis induced by drugs other than primaquine. If, indeed, there is a linkage of these hemolytic mechanisms and this bond can be traced to a defect in red blood cells, then a method for detection of such cells becomes a valuable laboratory procedure.

An excellent article[2] has reviewed recently the investigations into the defect in primaquine-sensitive erythrocytes. Although it is not entirely clear yet, it appears that they are deficient in glucose-6-phosphate dehydrogenase activity resulting in defective glutathione reduction in the older red cells of a cell population. The rapid fall in reduced glutathione which occurs when primaquine-sensitive erythrocytes are incubated with acetylphenylhydrazine appears to be an in vitro expression of the defect and serves as a means of differentiating sensitive from nonsensitive cells. This is the basis of the glutathione stability test.

This test has been used in various research laboratories and it was decided to investigate the feasibility of its use in a clinical laboratory. We were also interested in determining the incidence of the red cell defect in a random hospital population as well as trying to determine

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whether there was any correlation between its presence and clinical anemia or any specific disease pattern.

Methods and Materials

Reduced glutathione (GSH) was determined by Beutler's modification[3] of the Grunert and Phillips method.[7] In this method sodium nitroprusside produces a rose-colored complex with the protein-free filtrate of whole blood and the color is stabilized with cyanide. Certain modifications simplified the procedure without sacrificing accuracy:

1. The whole blood was anticoagulated with acid-citrate-dextrose (ACD) in the proportion of 4:1.[2]
2. The entire procedure was carried out at room temperature in an air-conditioned room. The temperature ranged between 22-25°C.
3. The equilibration period of 10-20 minutes was found to be unnecessary if the solution were mixed thoroughly by inversion before the addition of nitroprusside.
4. A Kahn shaker proved a handy substitute for the Burrell wrist action shaker and a Beckman model DU spectrophotometer was used to measure the optical density of the colored solution.

Glutathione stability was measured by comparing glutathione levels in blood before and after incubation with acetylphenylhydrazine (APH).

Two hundred GSH determinations and 200 GSH stability tests were performed on the blood of 51 adult Caucasians (38 male, 11 female) and 100 Negroes (45 male, 55 female). These persons were selected at random from hospitalized patients and blood donors. One patient who was found to have decreased GSH and instable GSH was given 30 mg primaquine daily for four days to verify the in vitro findings. Tests performed, other than glutathione stability, before primaquine was administered and within 24 hours after the last dose included: (1) hemoglobin determination by Cyanmethemoglobin method (2) hemoglobin paper electrophoresis in Durrum-type cell (Spinco) (3) autohemolysis[5] (4) plasma hemoglobin[6] (5) staining for Heinz bodies.[9]

Results

In order to establish normal values the data on Caucasians were grouped and the results are shown in Table I.

TABLE I

Results of GSH stability tests on 51 Caucasians.

	Mean GSH mg per 100 ml RBC	Standard Deviation x 2	Range mg per 100 ml RBC
Before incubation	63	±22	41-85
After incubation with APH	52	±20	32-72

The mean value for the percentage fall in glutathione level after incubation with APH was 16 percent with 2 S.D. ±14.

Applying these figures to the results on 100 Negroes, we found that in eight patients the glutathione level fell below 30 mg per 100 ml RBC after incubation with APH. Of these six (3 male, 3 female) fell below 20 mg per 100 ml RBC. This was a drop of over 60 percent in each and

the average results are shown in table II. The presenting diagnosis and hemoglobin, as well as the glutathione concentrations of each are listed in table III.

TABLE II

Results of GSH stability tests of 6 Negroes.

	Mean GSH mg per 100 ml RBC	Standard Deviation x 2	Range mg per 100 ml RBC
Before incubation	33.6	±13	20.6–46.6
After incubation with APH	9	± 9	0–18

TABLE III

Diagnosis, hemoglobin, GSH concentrations of 6 Negroes.

	Sex	Diagnosis	Hgb. mgs Percent	GSH mgs PER 100 ml RBC	
				Before Incubation	After Incubation with APH
L.J.	F	Atherosclerosis	9.7	38.5	15
L.R.	F	Thyrotoxicosis	14.6	31	6.5
H.H.	F	Ovarian abscess	13.6	31	3.5
H.L.	M	Blood donor	over 12.5	30.6	9.4
O.H.	M	Severe burn	9.2	25	5
L.M.	M	Polycythemia vera	15	45.5	14

The results on the remaining two patients (one male, one female) are shown in table IV.

TABLE IV

Results of GSH stability test on 2 Negroes.

	GSH PER 100 ml RBC		Percent Fall
	Before Incubation	After Incubation with APH	
B.K.	53	26.5	50
G.B.	45.5	21	53.5

Results of the test done on the blood of the patient who received primaquine are presented in table V.

TABLE V

Test findings before and after primaquine.

	Hgb.	GSH mg 100 ml RBC					
	Whole Blood gm Percent	Plasma gm Percent	Before Incubation	After Incubation with APH	Autohemolysis Percent	Hgb. Electrophoresis	Heinz Bodies
12/31/59	13.6		31	3.5			
1/ 1/60	13	0	37	9.35	1.9	60%A-40%S	none seen
1/ 2/60			Primaquine started				
1/ 4/60	11.8		20	1.0			none seen
1/ 5/60	10.4						
1/ 6/60	9.5	0	27.6	2.4	.98		none seen

Discussion

The falling whole blood hemoglobin level (table V) following primaquine administration to the patient who was found to have instable glutathione in the vitro confirms the presence of primaquine-sensitive erythrocytes. She was convalescing from a culpotomy which was performed 12/30/59. The fact that she was labeled a sickle cell carrier by hemoglobin electrophoresis makes the data from the hemolytic studies difficult to interpret. It is probable that the increased autohemolysis is due, in fact, to the sickle cell trait inasmuch as it was increased before primaquine was started. Although an increase in Heinz bodies is usually seen in patients with primaquine-sensitive cells, none could be demonstrated in this patient. It was interesting that Heinz body preparations on this patient showed greatly increased crenation of the red cells as compared with control preparations.

The normal values as shown in table I are in general agreement with those published. Beutler[3] used 121 Negroes plus 32 Caucasians and the average GSH was found to be 67.9 mg per 100 ml RBC and 64.9 after incubation with APH. In the 51 white females of Childs and coworkers[4] the average GSH after incubation with APH was 56.20 mg per 100 ml RBC.

There is no doubt that the six patients whose results are averaged in table II belong to the instable category for a fall to 20 mg or below after incubation with APH is generally accepted for this group. Repeat tests showed that while there is considerable variation at normal levels, there is very little at lower levels. No specific disease pattern could be found. The presenting diagnosis (table III) shows that they are a heterogenous group and the hemoglobin concentrations, which are probably more closely associated with the diagnoses, cover a wide range.

Although it had been thought that the glutathione stability test could sharply divide persons into two categories, a third group, intermediate, has been proposed.[4] This is the group whose GSH concentration falls between 22-40 mg per 100 ml RBC[4] or 20-35 mg per 100 ml RBC[2] and is composed mainly of females. Alving[1] has found that at these levels it is unsafe to predict in vivo primaquine sensitivity.

In this system the two patients listed in table IV are classified as intermediates but it is probable that they belong to the instable group. Two standard deviations predict 95 percent probability and it can be seen from table I that although initially the GSH of these is within normal range, following incubation with APH, it falls outside the normal limits. If these two can be placed with the other six, the mean GSH (table II) is raised to 37.2 mg per 100 ml RBC and after incubation with APH it becomes 12.2 mg per 100 ml RBC, but a drop of 50 percent can be used to distinguish stable from unstable GSH.

Because of the foregoing it is difficult to compare the incidence of positive results in this experiment with those of Childs[4] and Alving.[2] Discrepancies may be due either to differences in classification or to differences in patient population. It will be noted, however, that our incidence of percent glutathione instability in a population of Negroes (8 percent among males, 7 percent among females) compares well with that of Beutler.[2]

Conclusions

1. From a technical standpoint the glutathione stability test is easily adaptable for use in a clinical laboratory provided there is a sensitive spectrophotometer available.
2. The test provides a rather sharp differentiation between GSH stability and instability.
3. In one patient the GSH instability was shown to accompany primaquine-sensitive erythrocytes.
4. If it may be inferred from this and from the findings of others that this is true in all cases, then the glutathione stability test is a useful addition to other tests for hemolysis.

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AN EVALUATION OF SOME OF THE FACTORS WHICH ARE RESPONSIBLE FOR THE COLONIZATION OF THE NURSERY AREA WITH STAPHYLOCOCCUS PYOGENES*

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In the past several years a large volume of work has been done dealing with the problem of hospital infections in general, and staphylococcal infections in particular (Starkey 1956) and (Wysham 1957). There are many factors which play a vital part in the transmission of infectious agents, see (Letourneau 1958) and (Brown 1958) for summary. One of the most important factors is that of the carrier (Lepper 1955) (Clarke 1957) and (Hare and Ridley 1958). It is the usual custom to perform a quick survey during an epidemic to ascertain the identity of those who carry the offending bacterial strain and these individuals are then removed from the area. Frequently, such investigations are performed under the pressure of an epidemic and are of such short duration that we are unable to evaluate the pre-epidemic events which lead up to the state of general colonization and subsequent outbreak of infections. Without the unfavorable pressures which accompany an epidemic, real or apparent, we undertook an evaluation of the role that various groups played in the colonization of the nursery.

MATERIALS AND METHODS

Personnel Included in Survey:

All personnel who were permitted into the nursery area or who had contact with the babies of the nursery were included in this survey. All physicians concerned with the obstetrical service, laboratory and X-ray personnel, all personnel working on the obstetrical floor (nurses, students aides, housekeeping and maintenance personnel). In addition, all obstetrical patients and newborn infants were included.

Collection of Strains:

Bacteriological examinations were made at weekly intervals for a duration of four months (18 weeks) in all participants except the obstetrical patients and newborn infants. The obstetrical patients were cultured at time of admission to and discharge from the hospital. Babies were cultured only at time of discharge. Sterile saline moistened swab-cultures were obtained from the anterior nares of the participants. These swabs were streaked on to the surface of mannitol salt agar plates (Difco**) and incubated for 24 hours at 37° C. Mannitol salt agar positive staphylococci were further checked for coagulase activity using pooled human plasma which was diluted 1:5. All coagulase positive staphylococci were preserved on trypticase soy agar slants (BBL***) at minus 20° C until all collections were completed. More than 3,000 cultures were obtained which yielded approximately 700 coagulase positive staphylococeal strains.

Phage Typing of Isolated Strains:

Phage patterns were obtained on each of the isolated strains of coagulase positive staphylococci. The methods used for virus propagation,

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**Difco—Digestive Ferments Co. Difco Laboratories, Inc., Detroit, Michigan.
***BBL—Baltimore Biological Laboratories, Baltimore 18, Maryland.

titration and use were those of Blair (1958). The staphylophage battery included the following staphylophages: 3A, 3B, 3C, 6, 7, 29, 42B, 42D, 42E, 44A, 47, 47C, 52, 52A, 53, 54, 55, 70, 73, 75, 77, 79, 80, 81 and VA 4.

Antibiotic Sensitivity Patterns:

Antibiotic sensitivity patterns were determined for each strain using a panel of sixteen (16) antibiotic or chromotherapeutic agents. The disc was used employing Sensi-Disc (BBL) of the lowest commercial concentration of each agent. Mueller-Hinton Media (Difco) was used as the test medium. The panel of antibiotic substances used were as follows: Bacitracin, Carbomycin, Chloromycetin, Erthromycin, Furadantin, Mandelamine, Neomycin, Novobiocin, Oleandomycin, Penicillin, Dihydrostreptomycin, Tetracycline, Kantrex, Ristocetin, Vaneomycin and Triple Sulfa. The results of this portion of the investigation is to be reported at a later time.

EXPERIMENTAL RESULTS

Patients as Carriers:

There were 384 patients in the survey from which admission and discharge cultures were obtained on the mother and a discharge culture on the infant as shown in Table 1. It was found that 99 mothers (25%) were carriers of coagulase positive staphylococcal strains at time of admission. Sixty-two (63%) of these mothers lost the carrier state while being hospitalized. Thirty-seven (9% of the total) were still colonized with coagulase positive staphylococci at time of discharge. One-half (19) of these mothers, however, traded phage types during hospitalization. That is to say, they were admitted with one phage type and discharged with another. Thirty-seven mothers who came in negative had picked up a strain by time of discharge. There were 81 colonized infants at time of discharge. Our desire was to try to trace the possible of these staphylococcal strains. It is reasonable to assume that anyone having contact with the nursery or the infants could theoretically share in the colonization of these infants.

TABLE I—Distribution of Coagulase Positive Staphylococci Among Obstetrical Patients

Total Number O B Patients in Survey	Colonized on Adm.	Not Colonized on Adm.	Colonized on Discharge	Colonized with same Strain	Net Colonization	Percent
384	99	...	37	19	18	14
	...	285	37	...	37	

Others As Carriers:

Table II shows the distribution of carriers among the physicians, O.B. personnel, laboratory and X-ray personnel. We see that roughly one-half of the individuals are carriers at some time. The laboratory group had the highest incidence (63%) while the obstetrical group had the lowest (51%). Some individuals were permanent carriers of one phage type while others were permanent carriers but were harboring a variety of strains during the survey. Without respect to phage types, 10% of the laboratory and 5% of the obstetrical personnel were carrying coagulase positive staphylococcal strains on every examination.

TABLE II—Distribution of Staphylococcal Carriers Among Various Hospital Groups

	No. in Survey	Percent Neg. On Each Exam.	Percent Pos. At Least Once	Percent Positive On Every Examination
Physicians	39	46	54	0
O B Personnel	72	49	51	5
Laboratory	22	37	63	10
X-Ray	7	42	58	..

Strains Residing in Nursery at Onset of Survey:

At the onset of this survey there were five (5) different phage types of staphylococci in the nursery as indicated in Table III. We have excluded from this report all infants who were colonized with one of these strains at time of discharge.

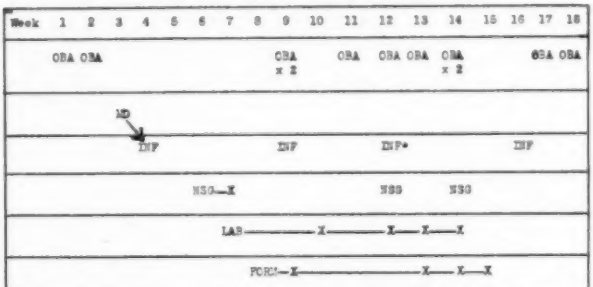
TABLE III—Infant Colonization with Staphylococcal Strains in Nursery at Onset of Survey

Strain	Discharged Infants Colonized With This Strain
Untypable	24
6/47/53/54/73/75/77	2
53/77/VA4	8
29/52/52A/80	1
52/79	2

New Phage Types Appearing During the Survey:

Of the nine new strains which were found in the discharge infants during the survey, we are able to see a positive correlation in four. In strain 44A (Table IV), the most common type brought into the hos-

TABLE IV—Distribution of Phange Type 44A

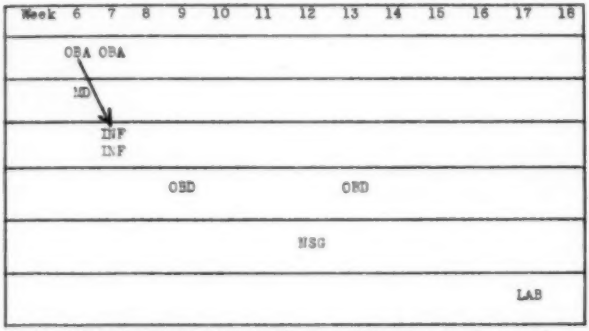


OBA - Admission Obstetrical Patient
OBD - Discharged Obstetrical Patient
MD - Physician in Obstetrical Service
INF - Discharged Infant
NSG - Nursing Personnel
LAB - Laboratory Personnel
FORM- Formula Room Personnel
• - Mother and Child

pital by obstetrical admission patients during the survey, we are able to follow the distribution of the strain quite effectively. This strain was brought into the hospital on weeks 1 and 2 by an admission obstetrical patient (ORA). On week 3 a physician delivered a child who subsequently became colonized with this strain. This strain was not found in any other personnel until two weeks later. At that time, week 6, it made its first appearance in the nursing personnel, a practical nursing student. This same week it is also found in a discharged obstetrical patient (OBD). Following week 6 there is a general distribution of this strain. We note that on week 12, both a mother and her child are discharged with this strain.

The second strain, 3C/6/42B/42D/42E/44A/52/53/54/75/77/79/80/81, was first introduced on week 6 (Table V) by an ORA. The child of this mother, and another child (the same week) were colonized with this strain at time of discharge. This strain was seen only sporadically after week 6 and no other discharged infants (NBD) were colonized with this strain.

Table V. Distribution of Phage Type
3C/6/42B/42D/42E/44A/52/53/54/75/77/79/80/81

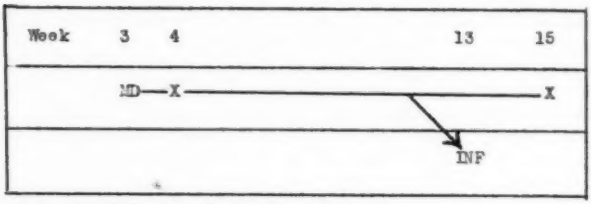


ORA = Admission Obstetrical Patient
OBD = Discharged Obstetrical Patient
INF = Discharged Infant
NSG = Nursing Personnel
LAB = Laboratory Personnel

Table VI shows the distribution of strain 44A/52/52A. This strain appeared in only two individuals during the survey. The first, and consistent appearance was in a physician in the obstetrical service. One infant, delivered by this physician, became colonized with this strain.

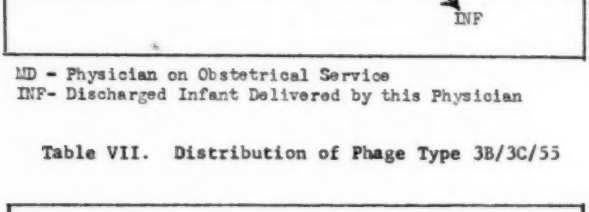
The last strain which made its appearance in an NBD and from which the likely source was evident was with strain 3B/3C/55. (Table VII). Even though this strain was found in nursing personnel on weeks 2 and 3, it did not reappear until week 17 in an ORA. The child of this mother was colonized with this strain at time of discharge. The survey was discontinued and it is not known whether this strain gained general distribution.

Table VI. Distribution of Phage Type 44A/52/52A



MD - Physician on Obstetrical Service
INF- Discharged Infant Delivered by this Physician

Table VII. Distribution of Phage Type 3B/3C/55



NSG - Nursing Personnel
OBA - Obstetrical Admission Patient
INF - Discharged Infant

Table VIII shows five other strains which were found in NBD but were not found in any other individuals in this institution.

TABLE VIII—Distribution of Phage Type 55

Week	3	4	9	16
	INF.	MAIN	LAB.	LAB.

INF. —Discharged Infant.
MAIN—Maintenance Worker.
LAB. —Laboratory Personnel.

Phage Types Found in Infants Only

42B/47C/52/52A/80/81	Week 3
44/52/70	Week 7
47/53/79	Week 12
55/77	Week 12

DISCUSSION

The role of the carrier in the transmission of infectious agents has been appreciated for many years. Indeed, the site of colonization of the carrier may play a very important role in the communicability of a

bacterial strain. We chose the anterior nares as the site to check as an index of colonization. While this site serves as well as any single site as an index, Hirst (1959) demonstrated several other sites which are colonized with staphylococcus in the newborn infant. Koch et al (1959) likewise demonstrated the desirability of multiple-site cultures for colonization studies. Lepper et al (1955) showed the distribution of coagulase positive staphylococcal strains among 217 persons in the Municipal Contagious Disease Hospital in Chicago. He showed that on a random sampling, approximately 25% of the group would exhibit staphylococcal carrier state. Upon repeated examination, the per cent carrying coagulase positive staphylococcus increased to 76%. He was able, also, to demonstrate the transiency of the carrier state. Sixty-six per cent of his group lost the specific carrier strain within one month. Our findings are in general agreement with Lepper's findings. However, by using a "closed" control area, the nursery, we were able to follow the introduction and distribution of the staphylococcal strains. Our group also showed an approximate 25% colonization rate at any one sampling. However, only one group, the laboratory personnel, reached as high as 63% (Table II). The per cent colonization in other groups was approximately 50 per cent.

In our group of 140 participants, we had only six individuals who were carriers 100% of the time. Only one of these individuals carried the same strain throughout the survey.

What is of considerable interest is the area from which these strains originated. In two instances (Table IV and Table VI) the obvious implication is that the physicians were responsible for donating the new strain to the infant. In two other instances (Table V and Table VII) most evidence points toward the implication of the admission obstetrical patient as the probable donor. The link is missing which would serve to elucidate the probable source for five other strains (Table VIII) which appeared in the discharged infants during this survey. In no case did we find the hospital personnel implicated in the original donation of a staphylococcal strain. It is reasonable, however, to assume that if the survey had continued for a longer duration of time, all groups would have been equally responsible for the colonization of the nursery areas.

CONCLUSION

It is the opinion of the authors that two important facets of nursery colonization problem have either been overlooked or given only minimal consideration. During the 18 weeks of the survey, there were nine (9) new strains of coagulase positive staphylococci introduced into the nursery. The data suggests that the donors of two of these strains were the attending obstetrical physicians.

Two more strains could be traced to the admission obstetrical patients as the prime suspect. In each instance the child of the colonized OBA became colonized. Four strains appeared only in the discharged infants and no possible sources of these strains were ascertained.

In this survey, the hospital personnel did not constitute an important group in the original colonization of the nursery but played an important role in the distribution of the strain throughout the hospital.

SUMMARY

A survey was conducted to study the role that various groups played in the colonization of the nursery with coagulase positive staphylococci. A total of 140 institutional personnel, which represent the obstetrical physicians, nursing, X-ray, and laboratory personnel, were examined at weekly intervals for staphylococcal carrier state. Approximately half of these individuals were carriers at some time during the survey. Six individuals were positive on each examination and all but one of these individuals carried different strains during the survey.

Three hundred and eighty-four (384) obstetrical patients were examined upon admission to and discharge from the hospital and the newborn infants were checked only at time of discharge. Approximately 25% of the admission OB patients were colonized with coagulase positive staphylococci at time of admission. There was a 14% colonization rate of the OB patients in the hospital. A total of 50 infants were discharged from the hospital while in a carrier state. Thirty-seven of the infants were carrying strains which were part of the nursery flora at the onset of the survey. Thirteen of these infants were carrying strains which made their appearance during the survey. No possible donors can be ascertained for five infants, each colonized with a different strain. The data suggests that two physicians were responsible for the introduction of two strains involving a total of five infants. Two admission obstetrical patients are implicated in the donation of two different staphylococcal strains involving three infants.

During this survey, no hospital personnel were found to donate a new staphylococcal strain to the nursery area but they are implicated in the distribution of these strains.

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HEMAGGLUTINATION IN HEPATIC DISEASE*†

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An impressive array of serologic tests utilizing the techniques of complement fixation, precipitin reaction, collodion particle agglutination, and hemagglutination has been used in the numerous attempts to discover a specific test for viral hepatitis.[1] Unfortunately, none of these has, up to the present, achieved the long-sought goal of specificity. Particular interest has been expressed in recurrent attempts to demonstrate agglutinins for a wide variety of erythrocytes in the serums of patients with viral hepatitis. The erythrocytes of rabbits, sheep, adult chickens, monkeys, and man have been used, but in spite of finding a variable percentage of positive results in patients with hepatitis, none of these tests has proven to be specific. The exact mechanisms potentiating hemagglutination have usually not been well defined, although in certain early studies the agglutination of sheep erythrocytes by the serums of patients in the acute phase of hepatitis was defined as being due to a heterophile antibody that was dissimilar from that found in the serums of patients with infectious mononucleosis and could be differentiated from it by absorption on guinea pig kidney.[2] A few years ago, Hoyt and Morrison[3] and, subsequently, Rubin et al.[4] described the agglutination of *Macaca rhesus* erythrocytes in the serums of patients with viral hepatitis, and the latter group interpreted this as being due to the specific effect of hepatitis virus. More recent observations of two other groups of investigators revealed that rhesus erythrocytes were agglutinated in the serums of 90% of normal persons or persons with acute infections other than viral hepatitis[5] and that there was no significant difference between the incidence of hemagglutinins for monkey erythrocytes in the serums of patients with hepatitis and in normal serums.[6] McCollum et al.[6] in addition found that serums from either normal persons or patients with hepatitis that had agglutinins for monkey erythrocytes also had equal or greater amounts of agglutinins for sheep erythrocytes, and the absorption of these positive serums with sheep erythrocytes removed the agglutinins for monkey erythrocytes, suggesting that the rhesus cell agglutinins are related in some way to sheep cell agglutinins that have been shown to be present in a low titer in a fairly high percentage of normal human serums.

More recently, interest has centered on the agglutination of day-old chick erythrocytes by the serums of patients with viral hepatitis following the extraction of these serums with lipid solvents. Three laboratories,[5-7] working quite independently, have utilized this test and have described essentially similar results, although some difference in incidence of positive tests has occurred that may doubtless be explained on the basis of difference in techniques. These results indicate that a large percentage of patients in the acute phase of viral hepatitis have in their serums hemagglutinins for day-old chick erythrocytes, while the serums

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of only a small percentage of normal persons, patients with chronic hepatic disease, and patients with other diseases contain these hemagglutinins. Of particular interest has been the fact that the serums of patients with obstructive jaundice do not hemagglutinate these erythrocytes, making this test of diagnostic value.

In the performance of any test, a number of problems are bound to arise. This has been true of the chick cell hemagglutination test for viral hepatitis, and it is the purpose of this paper primarily to discuss the performance of the test, the pitfalls to be avoided, and the interpretation of its results.

Materials and Methods

Preparation of Glassware

The presence of minute amounts of detergents has been shown to cause falsely positive hemagglutination. Therefore, all glassware is acid-washed (potassium dichromate cleaning solution), rinsed thoroughly in tap water, then three times in distilled water and three times in distilled demineralized water, and dried.

Preparation of Reagents

All reagents are made with distilled demineralized water or with triply distilled water, and are made fresh each month with the exception of the Alsever's solution, virus-adjusting diluent,* and borate saline** that are made fresh every two weeks and stored in the refrigerator. Molds tend to accumulate in the reagents, and such material should be discarded.

Preparation of Serum

The serum is freed from the erythrocytes by centrifuging twice at 2000 r.p.m. for 30 minutes. If the test is to be performed within the next three days, the serum may be stored in the refrigerator. If, however, the test is to be deferred beyond this time, the serum is frozen at once and stored at —20° C. or at dry-ice temperature. If serum is to be shipped, it should preferably be in the frozen state.

The serum (0.5 ml. or 1.0 ml.) is added slowly to 20 volumes of chilled acetone in a heavy glass centrifuge tube resting in an ice bath, with constant agitation of the tube followed by vigorous stirring with a glass rod. The tube in the ice bath is placed for 20 minutes in the refrigerator after extraction. During this time, the tube is agitated several times. The sediment is packed by centrifugation at 2000 r.p.m. for 5 minutes at 4° C. The precipitate is light and does not pack solidly enough to pour off the supernate so that the latter is siphoned off with suction from a water pump, using a side-arm flask as a trap. The sediment is again extracted with 20 volumes of chilled acetone and subsequently with 20 volumes of chilled acetone-ether (mixture of equal

* Virus-adjusting diluent:

per liter redistilled water { 100 ml. 1.5 M NaCl; 32 ml. 0.5 M Na_2HPO_4; 184 ml. 1.0 M NaH_2PO_4.

** Borate saline solution at pH 9.0:

per liter redistilled water { 80 ml. 1.5 M NaCl; 100 ml. 0.5 M H_3BO_3; 24 ml. 1.0 M NaOH.

Equal volumes of virus-adjusting diluent and borate saline solution mixed together give pH 6.0.

amounts) once, and then extracted twice in 20 volumes of chilled ether. It is dried thoroughly on a water pump, with careful attention to removing the ether completely, and reconstituted in 10 volumes of borate saline solution of pH 9.0, and stored overnight in an ice bath in the refrigerator for use on the following day. If testing is to be deferred, the dry sediment is stored at —20° C. or dry-ice temperature until needed. Tubes are stoppered throughout the procedure.

Preparation of Cells

Day-old White Rock chicks are anesthetized with ether and bled from the heart, using a 5 ml. syringe containing 2 ml. sterile Alsever's solution (autoclaved 10 minutes at 10 pounds pressure). The blood is pooled in Alsever's solution, using one part of blood to four parts of the solution. In this condition, the cells can be stored in the refrigerator for no longer than one week. On the day of the test, the cells are washed three times with 0.15 M saline solution, centrifuging for 6 minutes at 2000 r.p.m. at the last washing. The cells are then suspended in sufficient 0.15 M saline solution to give a 10% suspension. For the test, a 0.25% suspension of cells is used; this is standardized in a Klett photoelectric colorimeter (540 green filter) by making a 1:40 suspension in 0.15 M saline solution, obtaining the final volume of cells required by dividing the observed reading into the required reading (approximately 325). For the test itself, the original 10% cell suspension is converted to a 0.25% suspension by the addition of the necessary amount of virus-adjusting diluent composed to give a pH of 6.0 when added in equal amounts to the borate saline pH 9.0 (± 0.5). The 0.25% suspension of cells is made just before it is to be added to the tubes, and it should be added within a period of 15 minutes. If the test is to be large, it is better to make two different suspensions.

Technique of Hemagglutination Test

The tubes used in the test are precooled in an ice bath and kept in their racks in the ice bath throughout the test. A Cornwall pipettor is used in making the serial dilutions, and attention is paid to proper mixing and transferring. In testing unknown serums, eight tubes are ordinarily used, starting with a dilution of 1:10. Serial dilutions are made with 0.5 ml. borate saline pH 9.0 and 0.5 ml. of each dilution of reconstituted serum. 0.5 ml. of the 0.25% suspension of chick cells in the virus-adjusting diluent is added to each tube, leaving a final volume of 1.0 ml. A single cell control is used for each serum, using 0.5 ml. of borate saline and 0.5 ml. of cells in the virus-adjusting diluent. Tubes are shaken vigorously, incubated at 4° C. for 16 to 18 hours, and then read. Hemagglutination may be of the classic shield type or a rough, granular type. The occurrence of either type of agglutination is regarded as positive and a 1+ reaction at a titer of 1:80 has been accepted as significant. Negative reactions are characterized by a round, well-circumscribed button of cells on the bottom of the tube. Known positive and negative serums are included in each test. If the reactions of these serums differ by more than one dilution from customarily encountered reactions or if the cell control reveals agglutination, the whole test is repeated.

Care of Materials and Apparatus

It is assumed that every serum is potentially infectious, and therefore every effort is made to avoid contamination of the environment and personnel. The extractions of the serums are performed in a hood, and all equipment is autoclaved after use and before it is washed. Supernates from the extractions are evaporated from the side-arm flask on the water pump to get rid of the ether before it is autoclaved. To hasten evaporation, the flask may be placed in a container of hot water.

Results and Discussion

Attention has already been called to the fact that in contrast to the large percentage of patients in the acute phase of viral hepatitis that have hemagglutinins in their serums, serums of patients with obstructive jaundice do not agglutinate these cells. It is not known whether this actually means an absence of hemagglutinins in the latter serums or, as is far more likely true, that there exists in these serums an excess of inhibitors making hemagglutination impossible. Some support for this latter concept is furnished by the fact that the serums of patients with obstructive jaundice inhibit the hemagglutinins in the serums of patients with viral hepatitis. This serologic reaction is not likely to be a specific antigen-antibody reaction but rather the result of physiochemical changes in the serum. The fact that the chick cell hemagglutination test is positive in only a small percentage of normal persons, of patients with chronic hepatic disease, and of patients with a diversity of other diseases enhances its value as a diagnostic test.

In viral hepatitis, hemagglutinins appear early, usually within the first ten days of disease, and diminish in convalescence. How early they appear is unknown, although it is of interest that in experiments with volunteers and also in observations of persons exposed to hepatitis, it has been shown in our laboratory[7] and also by Morris and Nakamura[8] that hemagglutinins appear in the serums of persons infected with hepatitis virus during the incubation period and several days before the onset of clinical disease. It would thus appear that even though no specific relationship has yet been demonstrated between the potentiation of hemagglutination and hepatitis virus, there is no question about the fact that the acquisition of hepatitis is closely related to the appearance of hemagglutinins in the blood.

Results of testing large numbers of serums of patients with chronic disease of the liver, including hepatic cirrhosis, various infiltrative diseases of the liver such as metastatic carcinoma, lymphoma, tuberculosis, and long-standing chronic passive congestion, indicate that only a small percentage of such patients have hemagglutinins in their serums, and that also there is no measurable relationship between the degree of hepatic involvement and the presence or absence of hemagglutinins. Attempts to correlate the presence of hemagglutinins and changes in various tests of hepatic function revealed no correlation between their presence and abnormalities of any one of the following determinations in the serum: glutamic oxalacetic transaminase, cephalin flocculation, thymol turbidity, albumin, globulin, alkaline phosphatase, cholesterol, and retention of intravenously administered bromsulphalein. There did, however, appear to be some relationship between the occurrence of

hemagglutination and a pattern of alteration of a combination of these tests. Thus, hemagglutinins were not found in the serums of patients with intra- or extra-hepatic obstructive jaundice and the characteristic biochemical determinations commonly associated with these conditions, that is, increases in serum alkaline phosphatase and cholesterol with normal or slightly abnormal cephalin flocculation and thymol turbidity tests with or without notable increase in serum transaminase. This pattern was not absolute, however, and many patients with intrahepatic obstructive jaundice due to chronic cholangiolytic hepatitis, with increased amounts of serum alkaline phosphatase and cholesterol as well as severe abnormality of cephalin flocculation, thymol turbidity, and serum transaminase, had negative hemagglutination tests.

The fact that hemagglutinins have been demonstrated in the serums of patients in the incubation period of hepatitis raised the question, of course, as to whether this test might serve as a good screening procedure for donors in blood banks. Serums of nineteen donors who were presumed or proven to be carriers and the serums of nine donors who were suspected of being carriers of hepatitis virus were tested, and only one in each group had a titer of hemagglutinins of 1:80 or greater. Unfortunately, many of these donors were tested at considerable intervals from the time when they gave the blood that presumably caused hepatitis in the recipients. However, the evidence strongly suggests that the hemagglutination test as presently performed is not a good or reliable test for hepatitis virus carriers.[7]

Summary

(1) The chick cell hemagglutination test is positive in a large percentage of patients with acute hepatitis. It is uncommonly positive in the serums of normal persons and in patients with chronic hepatic diseases and a variety of other acute and chronic diseases. It is uniformly negative in the serums of patients with obstructive or hemolytic jaundice.

(2) The test is easy to perform, although it is somewhat time-consuming. If certain simple rules related to cleansing of glassware and freshness of solutions are observed, the results are highly reproducible.

(3) The mechanism of the test is not yet understood. Although the appearance of hemagglutinins is intimately associated with the acquisition of hepatitis, there is no evidence that the reaction is directly related to the presence of hepatitis virus. It seems more likely to be the result of alteration in physiochemical environment. In spite of our lack of understanding as to its mechanism, the test is of value in the differential diagnosis of the cause of jaundice.

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ABSTRACTS

THE E.S.R. IN A NEW DRESS

The Westergren method of determining the sedimentation rate forms the foundation of present-day clinical thinking. Other technics, notably that of Wintrobe, have fallen from favor for various reasons which are not discussed in the present article.

The many factors influencing the sedimentation rate are discussed in detail and with these in view, a modified Westergren method is presented. 2 ml. of sequestrenized blood are mixed with one-quarter of its volume of 3.8% sodium citrate and the 1 hr. fall determined in a tube which is hung from its top with a simple gimbal mounting.

The di-potassium salt of sequestrene was prepared in a 5% aqueous solution. Amounts sufficient to give a concentration of 1-1.5 mg./ml. of blood were dried at room temperature or at 45° C. for a few days. Dawson, J. B., Dept. of Medicine, U. of Edinburgh, Scotland. *British Medical Journal* June 4, 1960, pp. 1697-1704.

THE SIGNIFICANCE OF HOWELL-JOLLY BODIES IN RED CELL PRECURSORS

A series of sternal marrow films examined for Howell-Jolly bodies is reviewed. In this, the incidence in iron-deficiency and other states where B_{12} or folic acid deficiency could clearly be excluded has been compared with the incidence in conditions known to be associated with B_{12} or folic acid deficiency.

The presence of Howell-Jolly bodies in erythroblasts was found to be a useful aid in the routine interpretation of sternal marrow films. Where megaloblastic transformation is not well developed the finding of Howell-Jolly bodies provides helpful confirmatory evidence of the tendency and suggests the need for further investigation along those lines.

With regard to the origin of these bodies, it is likely that they are produced by abnormal mitosis in the late megaloblasts when single chromosomes or groups of chromosomes become detached, fail to take part in the formation of the interphase nucleus, and remain free in the cytoplasm as nuclear remnants.

It seems probable that disordered nucleic acid synthesis resulting from B_{12} or folic acid deficiency is the fundamental factor in the production of this abnormal behaviour at mitosis. Hutchison, H. E. and Ferguson-Smith, M. A., Dept. of Haematology, the University and Western Infirmary, Glasgow, Scotland. *Journal of Clinical Pathology*, 12, 5 1959, pp. 451-453.

THE SIGNIFICANCE OF BLUE POLYCHROMASIA

It is suggested that there is a difference in the significance of grey and of blue polychromasia in the adult peripheral blood and that the latter cell indicates a serious disturbance of hemopoiesis in the form of a megaloblastic, myelophthsic, or aplastic anemia. Davidson, E., Groote Schuur Hospital, Univ. of Capetown, South Africa. *Journal of Clinical Pathology*, 12, 4 1959 pp. 322-324.

Continued on Page 369

IMMUNOHEMATOLOGY OF BLOOD THERAPY*

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Introduction

The estimate[2] of 4,585,000 blood transfusions in 1956 for the population of the Continental United States was reported by the Joint Blood Council, Washington, D. C. Assuming 2.3 transfusions per patient (the figure employed for calculating purposes), the Joint Blood Council survey returns therefore indicate that in 1956 the persons who recived blood therapy totalled approximately 1,993,500.

In the opinion of many in laboratory medicine this annual rate has not declined since 1956—at least not appreciably, and some believe by individual observation and individually compiled data that it has increased. Let us discuss what is considered adequate pre-transfusion routine for the blood bank departments of any clinical laboratory.

It is generally accepted there are two main precautions:— to guard against the occurrence of a hemolytic reaction caused by blood group incompatibilities as well as against using a "sensitized" donor, i.e. one who has acquired blood group atypical antibodies.

Recommendations

I. ABO Blood grouping and Rh(D) typing should be done with *each* bleeding, not just reference to previous record. Bloods to be tested should be freshly drawn, not older than 24 hrs., and properly stored (refrigerated). REAGENT: In-date Anti-A and Anti-B Blood Grouping Serums, meeting the requirements of the National Institutes of Health, U. S. License. (This information is printed on Anti-Serum label.) METHOD: Room temperature saline tube test is the method of choice in our blood bank. Reverse typing or Serum typing, the use of pooled known A Cells and B Cells, should be a must on all donor bloods. Reverse typing may be helpful on a recipient with an unexplained incompatibility in crossmatching. Subgrouping of A Blood is not routinely done but is suggested when multiple transfusions are contemplated or there is a history of many previous transfusions or an unexplained reaction with previous transfusions.

II. **Rh Typing**

REAGENTS: (1) In-date Anti-Rh Typing Serum meeting same requirements as ABO reagent,
(2) Bovine Albumin 22% or 30%.

METHOD: Rh Slide Test technique is most generally used with the *routine* use of Albumin Control. (Donor or recipient cells 50% suspension in own serum, no saline, and to the suspension add 22% Bovine Albumin.) A view box containing a light bulb which will bring the temperature of the plate surface to 45-50°C—this is a necessary piece of equipment for Rh slide testing.

On recipients, only D typing is routinely done. All D negative donors must be tested for C and E and only if CDE negative, are classified as Rh "negative." Also on CDE negative donors the Du test must be performed. If

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Du positive, the donor is classified Rh positive generally, this is not applied to recipients, i.e. if D negative a triple negative (CDE) donor, blood is selected for compatibility testing.

The problem of false positives in Rh(D) typing is sometimes encountered. Some causes may be[12]:

1. Rouleaux formation due to protein abnormalities such as are found to occur in multiple myeloma. Saline washing of cells before testing is recommended. D Saline test tube technique is advisable. A high fibrinogen level in blood will cause strong rouleaux of red cells.
2. Auto-agglutinins: As in cases of acquired hemolytic anemias. These auto-agglutinins may be further demonstrated and confirmed by the performance of a Direct Coombs Test on the blood in question.
3. Evaporation or drying may be due to view box surface temperature above 50°C or insufficient quantities of cells to be tested. It is always good principle to follow the recommendations of the reagent manufacturers regarding quantities, time of observation, etc.

False D negatives are also encountered:

1. Cell suspension (to be tested) too weak
2. Hemolyzed Cells
3. Insufficient mixing of cells and anti-serum
4. Old blood
5. Failure to heat slide (of view box)
6. Outdated or contaminated anti-serum

N.B.—Contaminated serum may also give "false positive."

III. **Antibody Screening of Donor Bloods**

This may be done with group O pooled cells of known antigen content. Freshly drawn blood may be used or for greater convenience, some store blood at minus 20°C and thaw cells according to the method of Chaplin and and Mollison[6]. Pooled "test cells" are commercially available. This screening routine is some safeguard and in some instances, time saving.

IV. **The Universal Donor**

Much is found in the literature regarding the use of O Donor bloods for blood groups other than group O. Chaplin[5] uses the term the "dangerous universal donor" in the indiscriminate use of group O. Ervin et al[5] reports observations in vivo and in vitro behavior of iso-antibodies of immune type in group O blood inherent dangers and reports of hemolytic transfusion reactions following the use of such blood.

The following criteria are used by some for determining a safe O donor, to be used for a recipient of group other than O:

A. Determining the iso-agglutinin titer is not higher than 1:200 has been generally accepted. However, there are more and more conservatives who are using 1:64 as low titer O blood.

B. Bossom[4] reports a review of the literature indicates that iso-hemolysins as well as immune iso-agglutinins are regularly found in the universal blood donor which may cause hemolytic reaction if tranfused to a recipient. Therefore in addition to iso-agglutinin titer, an AB hemolysin screening test is employed by many. Only those donors who are negative for AB hemolysins are regarded as "safe."

C. Substances capable of neutralizing iso-agglutinins are sometimes added to the O blood before administration. Such as the commercially available product A and B substance.

Occasions may arise in any blood bank when ABO group specific blood is not available for a given recipient. Therefore it is advisable to have established criteria and materials available for such an emergency.

V. The AB Recipient is generally termed the "Universal" recipient. It is recommended[12] that if an AB blood is not available such a recipient be subgrouped with Absorbed Anti-A Serum or Lectin-A. Those recipients who are A_1B may be given A donor blood. The recipients who type A_2B, should be given B donor blood—for the obvious reason that the administration of A blood may induce the formation of Anti-A antibodies. Either A or B blood is preferable to O, the O blood having two iso-agglutinins instead of one.

VI. **For Transfusions**

A. The donor (or blood bank selected) blood should be the same ABO group as the recipient and for Rh negative recipients only type Rh (CDE or Du negative) blood should be used. In general Rh(D) positive recipients should be given D positive blood. The "convenience" use of Rh(D) negative blood for Rh(D) positive recipients is to be deplored because of the possible sensitization to the little c factor—which every CDE negative blood contains. This is to be particularly observed when transfusing women of child-bearing age or female children.

B. The finale of compatibility tests pre-transfusion is what I like to term the very, very important phase of blood banking routines. This is the compatibility tests of cross-matching the blood of donor and recipient. Such compatibility tests are of the greatest importance (and consequence) for recipients who have had previous transfusions, blood injections or pregnancies. Such conditions or blood therapy might have sensitized them to blood factors lacking from their own blood cells.

A number of different methods of carrying out crossmatching tests are in use. Antibodies (of recipient and/or donor) of any given specificity may occur in more than one molecular form of which the two most important are saline agglutinins (complete antibodies) and conglutinating agglutinins (blocking or incomplete antibodies). No single test will detect both these varieties of antibodies. Therefore, minimum requirements are:

(a) A saline solution medium—to detect the saline antibodies.

(b) A high-protein medium, such as 22% or 30% Bovine Albumin to demonstrate the blocking antibodies.

(c) The Coombs technique for certain non-agglutinating antibodies (with the two previous mediums) such as Kell, Lewis, Duffy and others.

Regarding blood samples of donor and recipient—prolonged storage of samples is to be avoided as loss of complement, necessary to complement depending antibodies, may occur as well as storage hemolysis of cells. If, on repeated use of donor blood pilot tubes, cell hemolysis is evident, saline wash a portion of these cells before use in compatibility tests.

The phenomenon of agglutination—the clumping of erythrocytes—demonstrates immunological reactions and when observed in crossmatch

procedures constitutes, detects, or reveals "incompatibility." Also in crossmatching tests, the hemolysis of erythrocytes (evident in the supernatant serum and by the decreased size of the cell button remaining in the bottom of a test tube) is another evidence of incompatibility. This may be due to increased red cell fragility or a hemolysin present and demonstrated.

The following is the procedure for routine cross match as performed at the Hospital of the Woman's Medical College of Pennsylvania, Philadelphia, Pa.

Note: For identification of cross match test tubes, "A" is used to designate the major side tubes and "B," the minor side.

Routine Cross Match Procedure

PATIENT'S BLOOD:

a. Collect blood using B-D Vacutainer, red top tube and immediately *label* with name of patient and hospital location.
b. Allow blood to clot, then rim clot, and centrifuge. Separate clot and serum, remove serum and put into small tube, labeled with name of patient.
c. Perform ABO and Rh routine typing.

STEP 1 **Cross Match**

Set up four properly marked test tubes (size 75 x 10), as follows:

MAJOR As
2 drops of donor's cells in 3-5% saline suspension
2 drops of patient's serum

MAJOR Aa
2 drops of donor's cells in 3-5% suspension in own serum
2 drops of patient's serum
2 drops 22% Bovine Albumin

MINOR Bs
2 drops of patient's cells in 3-5% saline suspension
2 drops of donor's serum

MINOR Ba
2 drops of patient's cells in 3-5% suspension in own serum
2 drops of donor's serum
2 drops 22% Bovine Albumin

STEP 2

Centrifuge all tubes for 30 seconds in Adams Serofuge. Then examine all tubes for agglutination and/or hemolysis.

NOTE:

We use the Test Tube Agglutination Viewer—as suggested by Dr. Louis K. Diamond, Children's Hospital, Boston, Mass. and available from Clay Adams, New York, Catalogue #A 2330.

Next step if Cross Match is compatible.

STEP 3

Incubate all four tests in water bath at 37°C for 15 minutes.

STEP 4

Centrifuge tubes for 30 seconds and examine for agglutination and/or hemolysis.

Next step if Cross Match is still compatible.

STEP 5

Take tubes As and Bs, wash the cell-serum mixture with tube fulls of saline for 3 washings. Centrifuge one minute between each washing.

STEP 6

At the end of the third washing centrifugation, the supernatant saline is completely decanted (Saline remaining should be approximately equal to cell button volume. Mix this residual saline and cells.) Add two drops of Anti-Human Globulin (Coombs) Serum. The tube contents are thoroughly mixed by tapping the tube. Wait 5 minutes and centrifuge for 30 seconds. Then examine the tube contents for agglutination. All units of blood for transfusion must be COMPATIBLE in *Step 2, Step 4, Step 6.*

All blood used in Cross Match—donor and recipient—are stored in refrigerator for at least 96 hrs. before discarding.

C. Jennings[9] reported the results of his survey on the present use of the minor cross match as a routine procedure. It interested us greatly to have the information that two thirds of the questionnaire returns indicated that it is essential to perform the minor crossmatch.

We are of the opinion that the detection by the minor (missed by the major) of an error in ABO group typing which would result in transfusing group O blood to persons of other groups and blood from other groups to group AB recipients—is in itself a safeguard—to use routinely. Also, the use of the Indirect Coombs on the minor side will demonstrate donor antibodies which could be "individual," i.e. specific for the blood group factors of the individual recipient. Why should anyone hesitate to include two more test tubes and the necessary time to do minor cross match in the battery of compatibility tests?

It is interesting to note that Levine[10] et al report that in a one year survey studying 13,000 blood specimens, approximately 1.4% contained demonstrable atypical antibodies. What guarantee or assurance have we that somebody, somewhere a sensitized recipient will *not* present himself as a donor!(?)

VII. Special Notes

A. How to handle the main non-specific agglutination encountered in Compatibility tests:

1. Rouleaux formation:
   This may be prevented in most cases by the addition of saline to the Cell-serum mixture. If the Indirect Coombs test is negative the interpretation of rouleaux is supported.

2. Cold agglutinins:
   This agglutinin may be found attached to cells or in serum or both. For ABO and Rh typing wash cells with warm saline (37°C), centrifuge at this temperature, and resuspend the cells in fresh saline. RH testing should be done with the washed cells using Saline Tube Test Anti-Rh Serum following the manufacturer's recommendations for the use of the Anti-Serum.

In cross matching, some recipient serum containing cold agglutinins will not show this phenomenon if tested at 37°C. Readings must be made promptly before mixtures reach 22°C (room temperature).

Taylor[11] contributed an excellent method for the absorption of the cold agglutinins. This is accomplished by mixing the recipient's serum with an equal volume of his washed cells (washing with saline at 37° C) and placing this mixture in a refrigerator. This absorbed recipient's serum is then used for the compatibility test.

3. Hyperproteinemia—Hyperglobulinemia[12]:
Blood from patients with high levels of protein exhibits increased rouleaux formation and with resulting may contain cold agglutinins. Agglutination of all cells including the patient's own cells. Perform ABO and Rh typings with saline washed cells. The indirect Coombs tests of the cross match procedure, if negative, establish compatibility.

4. Pan-agglutination[3]:
Two conditions may be the cause of this form of agglutination:
   (1) Erythrocytes of recipient are acted upon by certain bacteria and viruses in such a way that these cells are agglutinated by all human sera.
   (2) Also a sample of serum (donor or recipient) may become contaminated—obtain fresh blood samples.

B. For the recipient with auto-agglutinins—seek the least "incompatible" donor bloods. This may involve performing many cross-matches before success is attained.

C. The Life-Saving Emergency Transfusion:
Upon demand unmatched blood, group O, Rh negative is generally accepted as the safest. Ideally, the O blood is proven to be of low titer for iso-agglutinins and negative for A-B hemolysins. We request clinician to make the decision to use unmatched blood and he or his representative sign a release of the transfusion blood. It is advisable, circumstances and time permitting, to change to the recipient's blood group and Rh for subsequent transfusions. In less urgent cases, if the blood is issued (by request of clinician) before the compatibility tests are completed (with special reference to Coombs test of X-match) the results should be made available at the bedside of the recipient at the earliest possible time, so that the transfusion may be stopped if incompatibility has, in the meantime, been demonstrated.

VIII. **Exchange Transfusions**[1]

Many competent transfusionists routinely use group O (low iso-agglutinin titer (Rh negative blood for exchange of infants with erythroblastosis fetalis due to Rh incompatibility. Group specific blood may and should be used if the infant and mother are compatible in the ABO system. In cases of hemolytic disease of the newborn due to *only* ABO incompatibility, type specific blood is not used. Fresh group O, Rh positive is generally used for exchange of these infants.

The best blood for exchange transfusion is not more than 5 days old. Among the reasons for use of fresh blood are the necessity for preserving

a normal electrolyte balance in the infant and the desirability of avoiding injection of red blood cells rendered non-viable in storage.

Some transfusionists perform exchange transfusions with prior cross-matching procedure. In such cases the specificity of the maternal antibody should be known and the donor blood should be known to be free of antigens incompatible with the maternal blood. Others prefer to perform the compatibility tests with the mother's blood and that of the prespective donor, and this is considered a safer procedure. Blood group antibodies causing hemolytic disease of the newborn appear to pass from coated cells to fresh cells. Therefore, some exchange procedures are followed with "booster" transfusions and the same precautions should be observed when performing the compatibility tests.

IX. General Statements, and Precautions for Transfusion Service Departments

A. Identify recipient before drawing the blood sample for compatibility tests. A written request with full name of prospective recipient and hospital location should accompany the person with collecting equipment. There is no room for incompetence or carelessness.

B. Label all test tubes with legible writing or printing. It is a risk to leave the bedside of the patient without identifying the collected blood sample.

C. Regarding blood bank reagents, anti sera and anti Human Globulin (Coombs) serum, follow the manufacturer's directions, and frequently check the package directions—there may be a new recommendation for the use of the product. Unless otherwise advised, the anti-sera should be stored at refrigerator temperature, 2 to 10°C when not in use. Undue exposure to room temperature may cause loss of potency.

D. Use "Controls." Daily use is recommended. For ABO Typing, use known A, B, and O bloods (previously typed and so labelled). Likewise, for Rh typing, test anti serum with D negative and D positive bloods. For Coombs serum, sensitize any Rh positive cell with anti-Rh serum (in test tube) water bath for 60 minutes and saline wash cells 3 times, then add Coombs serum—agglutination is your positive control. For the negative control use an Rh negative cell and proceed as above. Always use albumin control for Slide Test Rh Testing.

E. All glassware must be scrupulously clean and free of traces of detergent. A test tube 75 x 10 mm is the suggested size to aid in reading the agglutination of cells. The use of flat-type tooth picks for the mixing of cells anti-Rh serum in the slide test is suggested. The disposable drinking straw (not waxed or dye colored) for the pipetting or transferring of cell suspensions and serum in the crossmatch, ABO and Rh typing is a great safeguard against contamination and saves much glassware preparation.

F. The physical set up of a blood bank working bench need not be behind closed doors, but we recommend an area free of unnecessary noise, disturbance and traffic.

G. Transfusion reports are required by some blood bank services. We solicit staff cooperation in the use of one. This form is a two copy (original and carbon) with snap out carbon. The technologist or technician fills out the patient's name, location, date or crossmatch, blood unit number (donor number) and then attaches the report form to the blood unit tag. When the unit of blood is signed out of the Blood Bank area, all our "paper work" is completed. The following is our transfusion report form:

TRANSFUSION REPORT

Patient's Name:
Location:
Date of X Match: Blood Unit No.
To be completed by physician giving transfusion. Original copy for chart, duplicate to be returned to Blood Bank within 24 hours.
Transfusion started by:
Patient's Temperature at Start of Transfusion: At End:
Time started: Time stopped:
Amount of Blood given: cc
Report of Reaction: Check ( ) if present
Chills Dyspnea Nausea Pain (located)
Urticaria Shock Add. Information
Signed by:

H. We believe it is advisable for every transfusion service to adopt uniform compatibility test procedures, i.e., all performing these tests—should use the same technique (around the clock). A department manual giving each required technique in full detail should be available in the blood bank area.

In Summary

It is the purpose of these discussions to stimulate thinking relative to the tremendous responsibility inherent in the operation of every transfusion service throughout the world. The discussions are far from complete in all details, but it is hoped they have some usefulness with special emphasis on compatibility tests, the immunohematology of blood therapy. "Blood is the mightiest of medicines—one out of every 20 transfusions saves a human life," says Paul de Kruif.[9] What a privilege to be an essential member of the team concerned with blood therapy.

Acknowledgment

I am deeply grateful to Dr. Mary McKee Porter, Clinical Pathologist, and Director—Clinical Laboratory, Hospital of the Woman's Medical College of Pennsylvania, and Chairman, Department of Clinical Pathology, Woman's Medical College of Pennyslvania, Philadelphia, Pa.—for her very generous editing and helpful recommendations in preparing this manuscript.

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THE BODIAN STAIN: ITS USE AND METHOD**

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There are many methods used to demonstrate nerve endings and nerve fibres in tissue. A number of them are modifications from the original Bodian stain. (3) They have probably been successfully worked out but those who do special stains, realize there are occasions when a method is followed very closely and still fails to give good results. The manner in which a technologist interprets a procedure has some bearing on the outcome of any stain. In doing a stain there are certain basic principles to be followed but modifications can be made. They generally consist of either a decrease or increase of timing periods, the substitution or omission of dyes or chemicals. This is sometimes done in order to work out a method adaptable to the geographical area and the individual laboratory.

The Bodian stain wasn't very successful in all parts of the country from 1936 to around 1940. During the second world war, the protein silver wasn't as pure. It contained more foreign material than previous years. Apparently this had an effect on the success of the stain, because from that time on, results proved more satisfactory. (4) There is still some difference in the manufacturing of it. The protein silver, known as Protargol-S put out by Winthrop Stearns* has proven the best. The original Bodian stain (3) and modification (1) is not difficult to follow but there are problems that arise when one is attempting this stain for the first time. This presentation of the Bodian stain with some modifications may aid a technologist in carrying out the technique to a successful ending.

The fixative for this stain is of first concern. The use of 10% formalin is satisfactory if the PH is kept near 7. Buffered formalin is used in this institution and is found to give good results. Bouin's solution has also been mentioned in conjunction with this stain and can be substituted for 10% formalin.

Method

The sections for this stain are fixed and dehydrated through alcohol and clearing agents of choice, infiltrated with paraffin and embedded. The sections are cut at 7 - 8 microns. The slides are allowed to dry in the oven at 58° — 60° C for half an hour. This will help to prevent them from coming off during the staining process. The use of celloidin to keep the sections on is not advisable as it will inhibit staining if not properly applied. After the paraffin is removed with xylols, the slides are passed through graded alcohols and then rinsed well in distilled water and placed in the Protargol S.

The method for the preparation of the 1% protein silver solution must be followed closely. The amount measured must be sprinkled on top of the water heated to 37° C and allowed to dissolve without being disturbed. (1) If the protargol is stirred into the water, it will form a sticky mass

* Winthrop-Stearns Inc., 1450 Broadway, New York 18, New York.

** Received for publication January 1960.

and become very difficult to dissolve. The prepared solution is poured into a coplin jar containing 6 grams of copper shot. This is to prevent the slides from becoming too heavily impregnated with silver therefore losing their detail. A small piece of gauze must be placed over the beads before placing the slides in the jar. If the slides are not protected in this manner dark brown streaks will develop. Coating the copper shot with a thin solution of celloidin will also serve the purpose.

The slides are kept in the protargol at 37° C for 48 hours. Some methods state 12-48 hours. (1) Less than 48 hours may be alright for some tissue but to be certain of a sharp detailed picture, leave them in for the full time. After removal from the protargol the slides are thoroughly washed three or four times in distilled water. This is very important as flocculation will develop.

A reducing solution is used next in the procedure. It also varies in different methods. The original reducing solution of Bodian (3) or Foley's modification (2) is very satisfactory but requires time to prepare. Reducing in a 1% hydroquinone in a 5% formalin (made fresh) for 10 minutes, works very well. The hydroquinone used should not exceed a year and a half in age. After reducing, the slides are again washed thoroughly in distilled water.

Remove the yellow color caused by the reducing solution by toning for 5-10 minutes in 0.02% aqueous solution of gold chloride with 20 drops of acetic acid added. (2) This gives a better toning with less loss of detail than with the 1% gold chloride. The timing varies in methods but toning until the yellow color is completely removed is sufficient.

The slides at this point are washed thoroughly in distilled water and developed in a 2% aqueous solution of oxalic acid. Here they must be watched very carefully. If the sections are in the solution too short a time, details will be missed and a blue-black color will develop. Over exposure will result in a reddish brown color. (4) After one minute of developing one must start looking at the slides under the microscope. As soon as the blue-black color starts to change to a reddish brown, rinse the slides immediately in distilled water. This usually takes around two and a half minutes. When staining muscle tissue for striations it may take longer. Use a control with this type of tissue. To remove the silver salt residue from the sections place them in a 5% aqueous solution of sodium thiosulfate for 5 minutes, followed by thorough rinsing in distilled water.

No counterstain is needed but if one is desired, a Van Gieson gives an excellent differentiation. (4) It only requires the placing of the slides in picro-fuchsin solution for 30 seconds. The slides are blotted dry and placed in absolute alcohol and cleared in xylols. They are then mounted in Permount.

Results

The nerve fibers, neurofibrils, a blue black; nuclei variable: Collagen takes on a greyish blue shade. If a Van Gieson was used for the counterstain, the nerve endings, nerve fibers, and neurofibrils stand out a deep blue black with the surrounding tissue shades of yellow and pink. Muscle stains a greenish yellow with striations a darker shade.

Summary

The Bodian stain with some modifications to aid the beginner is described above. This stain gives a very excellent picture of the nervous tissue. The most important point to remember is to procure a protein silver that is satisfactory. If poor results are obtained, first investigate the protein silver. Developing with oxalic acid has also a great deal to do with a satisfactory result.

A technologist should have little difficulty in obtaining an excellent picture of nerve endings and nerve fibers with the above points in mind and some histological understanding of nervous tissue.

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ANNOUNCEMENT

The twelfth annual postgraduate course in MEDICAL TECHNOLOGY will be presented on January 23, 24, and 25, 1961 at the University of Kansas Medical Center, Kansas City, Kansas.

Outstanding guest speakers will discuss the recent advances in microbiology, exfoliative cytology of the digestive tract, hematology, spectrophotometry and chromotography. Registrants will have an opportunity to participate in several of the workshops scheduled each afternoon.

The registration fee for the three days is $15.00. A program announcement may be obtained by writing to the Department of Post-graduate Medical Education, University of Kansas School of Medicine, Kansas City 12, Kansas.

SYMPOSIUM*

RESEARCH AND THE SCIENTIFIC METHOD IN MEDICAL TECHNOLOGY*

INA LEA GALLAHER, B.S., B.A., M.T. (ASCP)

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INTRODUCTION

Research—is this the stepchild of Medical Technology and ASMT? Why? We all know that research is necessary for advancement. In fact it has today become necessary for survival. Yet we as members of ASMT are doing little to further this work. We have allowed our Research Committee to remain idle and become stagnant when it should be the most active in the entire organization—functioning in close contact with all the other committees. Despite this inactivity during the past few years, the committee has been maintained because the members realize that it is an integral part of our society and that it is essential it now becomes the working committee that it should be.

To explain our disinterest in research we have only to go to the routine laboratory. Each day as specimens are collected, they are routed to the indicated department. Routine procedures are run as dictated by the laboratory director. The work load and pressure are constant. The work must get out—the report must be made as soon as possible to the clinician through the director of the laboratory. Between the actual bench work and report writing, little time remains for creative thinking. All too often our laboratories are short handed—we "double up." Research "desire" sinks to the bottom of our "need" list. Research is pushed back for tomorrow—a tomorrow that never comes. This attitude —perhaps created by necessity—is carried from the laboratory to our professional organization and we who should be striving constantly to promote research, are instead sitting back and watching others progress —while we, doing nothing *regress*.

How are we going to accept this ever growing challenge? Only by standing up and facing the situation that has been forced upon us and insuring that time and effort be set aside for creative thinking. How can we be assured that this time is available? There is only one answer to this—proper organization of thoughts and work. Research must be included as a vital portion of our every day effort. We would not refuse to do a blood chemistry or a serology simply because we were busy. Why then would we refuse to do another procedure that is just as important?

First, we must decide the project we wish to develop. Alert all personnel to be on the lookout for any specimen that should be included in the study. When one is found, merely add another determination

* Read before the Workshop on Research and the Scientific Method in Medical Technology, 28th Annual Convention of ASMT, Atlantic City, New Jersey, June 1960.

request to this and thus it is included with all the other procedures. Once we have done the work, we have merely to assimilate the data. This is an area where help can be available and where certainly we can expect the cooperation of everyone in our laboratory and laboratory community both in making time available for presentation of the data and in offering time and help.

The research committee this year is going to define the work that must be done by the state and local committees to become active and incite action in the people who really are going to do the research. It will be the duty of the state committees to work with these people who are curious and interested in acquiring new knowledge. We can help these researchers to develop their ideas and get them into presentable form—rather than to have just a collection of figures.

The following papers will stimulate interest in research as it pertains specifically to the profession of Medical Technology and how to conduct this research properly.

WHY RESEARCH IN MEDICAL TECHNOLOGY*

RAPHAEL COHEN, Director,
General Diagnostics Division, Warner-Chilcott Laboratories, Morris Plains, New Jersey

Importance of Research to the Service Technologist

The clinical laboratory field today has caught up with the pace of technological advance of American science. Scientifically, it's more fluid today, more subject to change, innovation, and progress, than at any other time in its history. And there are sound reasons for this current state of dynamism. For half a century or longer, many of the procedures in use in the routine clinical laboratory have undergone no significant change until now—for want of concerted scientific attention on their possible improvement in the light of more recent knowledge of both medical physiology and basic physics and chemistry. As illustrations, the red cell count and white cell count were introduced into use before the turn of the 20th century. The Kjeldahl procedure for nitrogen determination was introduced into use in the 1870's and the Folin-Wu Glucose method more than 40 years ago. The clinical laboratory, of course, is essential to the practice of medicine today and the practice of medicine, by its very nature, must be conservative in the finest sense of that word: resisting change for the sake of change alone, because of the enormous responsibility the physician bears for patient welfare, but never resisting change because of fear, born out of ignorance, when the procedure suggested offers significant improvements over what has been available before.

As director of an organization dedicated to serving the field of Medical Technology by effecting change where change is required, an educated, research-oriented group of medical technologists is important to me because it will insure that the fruits of *our* patient-oriented research will be at least tasted, and judgment made on the basis of the patient's and the laboratory's need, rather than emotion. Similarly, as responsible and ethical individuals, who play our role in service to humanity according to rules of conscience, we hope for a similarly well research-oriented group in the laboratory who will be able to evaluate, again without emotion, the techniques being advocated by fly-by-night operators who often bombard the laboratory with worthless materials.

The medical technologist, therefore, who is not involved in research projects in daily work, who is not teaching, who is concerned only with service to patients, requires knowledge of those habits of thought, those disciplines of attitude, and those constant qualifications in drawing conclusions which are the tools of the research scientist. The practicing technologist requires these just to keep up responsibly with the changes occurring in the professional field of Medical Technology, to be able to evaluate each new product, each new technique, each new concept by applying the age-old questions:

WHAT is It?
WHO says so?

* Opening Address: Workshop on Research and the Scientific Method in Medical Technology, 28th Convention of ASMT, Atlantic City, New Jersey, June 1960.

WHY does he say it? Does he have the data to substantiate his statement and are his data valid?

WHERE was the work done? Is the institution of sufficient stature so that complete objectivity is assured?

HOW was the work done? Is sufficient information given on methods so that I can repeat the study to confirm or invalidate what's claimed?

WHEN does this technique, this concept, this product, become sufficiently important to my goal of professional service that I become obligated to look into it?

These attitudes of thought are important to the service technologist, also, as a representative in a local community of a profession to which you want to draw others.

Importance of Research to the Technology Recruit

The profound shortage of well-trained technologists has been made a matter of extreme concern to everyone with any professional contact with the laboratory. Your own society, the American Society of Medical Technologists, has taken a lead in efforts to recruit the young, the bright, the dedicated, the compassionate scientific worker to your profession. Yet, the early drop-out rate is quite high. Professional opportunity is felt to be one reason for this turnover.

Isn't it true that many youngsters are drawn to the thought of medical laboratory work by the mental image conjured up by the word "laboratory": a place, according to the unabridged dictionary, devoted to *experimental study* in a branch of natural science; that is, a place where research is conducted. For the young recruit, drawn by dreams of participating in original contribution, the practicing professional service of the routine laboratory can spell disillusionment. The drama of Louis Pasteur and of Madame Curie is often quickly shorn of its glamour by the reality of repetitive procedure.

The provision of facilities for research in the student program is possible and is worth encouraging. It can go far to attract further and to hold more lastingly those very individuals who can make the original contributions that will add so much to the stature of this profession.

Research-mindedness as an integral part of your educational programs thus, very selfishly, becomes another tool to lead our brilliant young into your profession. But what of the other advantages that research-mindedness holds for the *teachers* and *teaching supervisors* of these schools?

Importance of Research to the Teachers of Medical Technology

The research attitude, as Dr. Arnow will discuss more deeply in his discussion of the scientific method further on this morning, holds that no scientific truths are fixed, not subject to change—that facts, as we know them today, may become untrue in the light of the newer facts we learn tomorrow. As teachers, your work requires not only the transmission of *information,* but even more significantly, the transmission of *inspiration, motivation,* and a set of attitudes that will result in your students becoming worthwhile members of your profession. The research you

perform becomes a significant part of the attitudes about methods and facts which you impart to your students. Shall we tell a student: "This is the way to determine the molarity of a solution?" Or shall we tell him: "This is the most efficient way of determining the molarity of a solution according to the information available today."

There are, of course, extremes to be avoided. In many of our universities and medical schools, no individual is considered fit for a full professorship without engaging in conspicuous research activity and prolific publication. As our shortage of excellent college teachers increases, many civic leaders and even educators have raised the question of whether or not skill in communication of concepts may not be more important than the performance of actual research. On the other hand, there are those who carry this attitude so far as to feel communication skills are all that is required. There is, of course, a happy medium.

I believe many of us interested in the relationship between research and education, as it applies to medicine and its allied sciences, would find of value the symposium published on this topic in the May 23, 1959 issue of the Journal of the American Medical Association. Each of the papers in the symposium carry some quotable items of particular pertinence to this audience. The first paper in this symposium, entitled: "RESEARCH AND TRAINING," by Conrad A. Elvehjem, Ph.D., President of the University of Wisconsin, includes the following:

> "Teaching can best stimulate the mind of the student when the mind of the professor is alive and questioning, as someone has put it, "when he is continually seeking new knowledge that will justify or correct his earlier impressions, when he kindles in the mind of the student by his own fire that lamp of truth which reveals all knowledge as an age-long and immortally unending search.
>
> "My first point then is that teaching must be a continual process of education for the teacher and that it can be carried out satisfactorily only when he constantly tries to enlarge his knowledge and test his ideas by an experimental method, which is research. I think that most of us can agree on that point."

And, on student research, Dr. Elvehjem quotes the Lebanese poet, Kahlil Gibran, as follows:

> "'A teacher does not bid you enter the house of his wisdom, but rather leads you to the threshold of your own mind.' This is the process of joining learning. This is student research."

The second paper in this symposium, by Dr. William Hubbard, Associate Dean of New York University School of Medicine deals with Research Orientation and Methods. Dr. Hubbard states:

> "As recently as 25 years ago, only a handful of the medical faculties in the United States provided, in the basic medical sciences, an opportunity for more than a passive participation of the student in a series of didactic exercises and laboratory manipulations. The latter were commonly carried out from a detailed recipe book with the quality of the effort being judged largely by its conformity to a predictable outcome. In subsequent years there has been an exponential increase in knowledge pertinent to medicine, arising largely from research initiated in the areas of the basic medical sciences. The volume of important information is now so large that it is a manifest impossibility to present it in a comprehensive didactic fashion in the time allotted. As a result, an increasing degree of selectivity has to be exercised by teachers in choosing the material which they will present. The rapidity with which new information develops has now made it quite obvious that it is at least as important for the

student to understand the process by which scientific data are reevaluated and accumulated as to master the momentary common store of knowledge."

And, about physicians, but I believe its application to Medical Technology cannot be questioned:

(They) "...must have learned habits of critical inquiry and continuing scholarship that will allow them to remain creative members of a learned profession rather than simply practitioners, albeit skillful ones, of a vocation. They must be critical of their own judgment and decisions, discerning about the validity of what they read, skeptical of final conclusions, and anxiously concerned about the individuality of the problems to be solved."

And my final quotation from Dr. Hubbard is as pertinent to your recruiting efforts for excellent teachers as it is for the similar situation in medicine:

"Unsolved problems of crucial importance concerning man and his diseases are still to be approached in all fields of medicine. Because of this, there is an increasingly urgent need for talented persons to take up careers of investigation and teaching in medicine. At the present time, the need is not being met. On the contrary, the number of medical school graduates in training for such careers seriously threatens, through shortage, the continuing progress of medicine. *The spirit of adventure that is inherent in a search for understanding* must be made available to the student if he is to sense the satisfactions of an academic career. Through participation in research activities *he can personalize the growth of knowledge* rather than look on it as some extraneous phenomenon."

Although many of the other articles in the symposium offer thoughts as pertinent as these, our time this morning permits us to quote only one more, the article by James A. Shannon, Director of the National Institutes of Health, on the "Maintenance of the Synergistic Relationship of Education and Research in Medicine." Synergism, of course, is a process of living together to the mutual benefit of both. I quote:

"A third area for concern in the question of medical teaching and research has to do with the experience and contributions of physicians, scientists, and teachers who have completed their formal training but for whom education and research are a never-ending process. It is of the utmost importance that the mechanisms for interchange and refreshment be strengthened and expanded to permit the individual to advance in his own and related fields, for medicine has changed dramatically in the past 20 years and certainly will continue to do so. Teaching must be of a nature that permits subsequent growth of both student and teacher."

Where Do We Start?

Naturally, not every one of us is going to become a research worker. Many of us will have no inclination to do so. Many of us, whatever our inclination, just do not have the time. And, of course, many of us in this audience today are already deeply involved in research of the most significant kind.

To all of us, however, the provision of an atmosphere which encourages research at the level of the professional Medical Technologist—an atmosphere in which research is considered important, and which enables each of us to grow in stature, is something which can be made possible by our attitudes.

In The Meantime

Naturally, developments such as these do not take place overnight and require the courage, interest, and dedicated effort of many people in

your profession and your professional society before coming to full flower. In the meantime, each of us presently functioning in the laboratory can assume as much of the research attitude as our time, our training, and our reading permits us.

ABSTRACTS

(Continued from Page 350)

QUANTITATIVE DETERMINATION OF GASTROINTESTINAL BLEEDING USING CR^{51}-LABELED RED BLOOD CELLS

Patients' red blood cells were tagged with Cr^{51} and reinjected into the patient. 24 hr. stool specimens were collected for a period of 7 days in most instances. The stool was then homogenized and its radioactivity determined by a well-type scintillation counter. A daily blood sample from the patient was also counted and the radioactivity of whole blood was determined. From this value plus the total stool volume, the amount of blood lost in the stool/24 hrs. was calculated according to the following formula:

$$\frac{\text{Radioactivity count/24 hr. stool specimen}}{\text{Whole blood radioactivity/ml.}} = \text{Total volume (ml./24 hrs.) of blood lost in the stool}$$

23 patients were studied. There was a lack of correlation between the presence of occult blood as determined by a benzidine test and the amount of blood demonstrated by the Cr^{51} technic. It has been shown that application of this radioactivity method gives reliable information as to the amount of blood lost through the gastrointestinal tract. Izak, G., Stein, Y. and Karshai, A. Dept. of Medicine B and the Hematology Laboratory, Hadassah-University Hospital, Jerusalem, Israel-Jordan. *American Journal of Digestive Diseases*, 5, 1 1960, pp. 24-31.

MEASUREMENT OF RED BLOOD CELL VOLUME WITH THE ELECTRONIC CELL COUNTER

These frequency distribution curves differ from the original red cell distribution of Price-Jones in that they describe cell volume instead of cell diameter.

In the Coulter counter, control knobs are provided for the aperture current and for the threshold circuit. Increasing the aperture current causes a greater strength and density of current through the orifice and smaller particles will be counted, inasmuch as they will cause a greater voltage change at any threshold.

The signal amplification, or threshold control is set at a fixed low position for total red cell counts and turned to successively higher settings to plot threshold curves. The count is constant at low thresholds and drops as the threshold rises. The threshold scale supplied with the instrument has arbitrary units that range from 0-100.

The aperture current setting was kept at 4 throughout the study.

Values of mean cell volume in the specimens studied were 39.5-154.7 μ^3, including those of various animals.

Each instrument must be calibrated individually because settings will vary from one machine to another.

The relation of the cell volume to readings of the threshold scale was determined. Grant, J. L., Britton, M. C. and Kurtz, T. E., Medical Service and Dept. of Research, Veterans Administration Hospital, White River Junction, Vermont. *American Journal of Clinical Pathology*, 33, 2 1960, pp. 138-143.

THE SCIENTIFIC METHOD*

L. EARLE ARNOW, Ph.D., M.D., President

Warner-Lambert Research Institute, Morris Plains, New Jersey

A. Philosophical Considerations

Introduction—One dictionary defines science as "accumulated knowledge systematized and formulated with reference to the discovery of general truths or the operation of general laws." In other words, the scientist assumes that natural phenomena are dominated by a set of "laws of nature"; he attempts first to guess what these laws are, and then tries to prove or disprove the validity of his guesses.

Although the title of this talk is "The Scientific Method," I do not believe there is in truth such an entity. If I must none-the-less offer a definition, it is this: The scientific method is the way in which scientists go about doing their everyday work. Dr. P. W. Bridgman, Professor of Natural Philosophy at Harvard, says it this way: "The scientific method, insofar as it is a method, consists in nothing more than doing one's damndest with one's mind—no holds barred."

It is easy to trace out the general procedures that have been used in formulating the present list of laws of nature. As we shall see, the difficulty lies in trying to generalize when we are not making a Nobel-prize-winning discovery, but rather are doing an experiment, or are carrying out an analytical procedure, in the laboratory.

How did Einstein go about formulating his theory of relativity? First he made a careful study of the observations and theories of other scientists. In doing this, he noticed that either the theories then in vogue were erroneous, or the observations were inaccurate. He assumed that the older theories were not correct, and set up an hypothesis (the special theory of relativity) that did explain the observations that had been made. This hypothesis, or scientific guess, made it possible for Einstein and others to predict the outcome of experiments not yet done. As these experiments have been carried out over the years, the predictions have been found to be true. This, of course, has strengthened our belief in the usefulness of the theory of relativity. There is no real doubt in my mind, however, that observations that are not consistent with this theory will be found in the future. When that happens, some genius, probably as yet unborn, will formulate a new hypothesis that will lead to still other verifiable predictions.

It is obvious that there are very few scientific truths. Instead, science progresses from one hypothesis to another. Of course, some hypotheses have persisted so long, and seem so obvious to us, that we are willing to call them laws of nature—and, in fact, are willing to believe in their absolute truth. For example, we cannot prove that an apple will fall in the direction of the earth if we shake it from its tree—but nobody really doubts that it will. Pouring a solution of sodium chloride into a solution of silver nitrate *may not* result in the formation of a white precipitate of silver chloride—but every chemist *knows* it will.

Induction and Deduction—The steps in the advancement of scientific knowledge, then, are:

* Presented before Workshop on Research and the Scientific Method in Medical Technology, 28th Convention of ASMT, Atlantic City, New Jersey, June 1960.

(a) The making and systemization of observations of natural phenomena.
(b) The sorting of these observations into those that are related to each other and those that are not.
(c) Making an hypothesis by means of *generalization* or *induction*. If, for example, one of you has found sugar to be present in every sample of human blood you have examined over, say, the past ten years, it seems fairly safe to generalize—that is, to conclude that the blood of *every* human contains sugar.
(d) Making predictions, known as *deductions*, based on the hypothesis. If these predictions can be proved to be correct, the hypothesis, though not proved, is strengthened.

I once had a teacher who explained induction and deduction in the following way. If I am given a series of twelve diamonds, and if each time this happens I discover that a jeweler will give me a great deal of money for the gem, I probably will decide by *induction* that all diamonds are valuable. Having reached this conclusion (or hypothesis) when next I see a diamond, I will conclude by *deduction* that this *particular* diamond is valuable.

Authority vs. Experimentation—The old Greek philosophers did not do experimentation. Instead, they imagined ways in which natural phenomena could be explained. These imaginary explanations were accepted as truths, sometimes persisting as such for centuries. In contrast, the scientist rejects *authority*, and relies instead on experimentation. It is true, of course, that he cannot do every experiment himself, and hence that he has to accept as valid a great deal of the work of other scientists. Still, he reserves the right to repeat the experiments of others, and to decide for himself whether or not their conclusions are valid.

As a matter of fact, the body of science is made up of the collective judgment of scientists. There are very large areas of agreement in spite of the non-authoritarian attitude of scientists. Perhaps this is the best evidence for the validity of scientific methods.

Of course, the fact that most scientists in a given field are in agreement about some aspect of science does not constitute proof of its validity. For example, some modern textbooks of nutrition still discuss the milk-clotting enzyme, rennin, as though it occurs in human gastric juice. As a matter of fact, it has only been proved to occur in the fourth stomach of the calf.

Nature of Hypothesis—The hypothesis is such an important tool of science that some more things should be said about it. It may be a simple generalization of a few observations. On the other hand, the intuitive formation of far-reaching hypotheses has been the hall mark of scientific genius throughout the years. Newton's laws of motion, the atomic theory, the theory of ionization, the quantum theory, the theory of relativity—all these resulted from the educated guess of a genius.

Sometimes, indeed often, more than one hypothesis to explain a given set of observations can be devised. When this happens, scientists invariably choose the simplest one for exploration. It cannot be *proved* that this is the correct course to follow. It would be difficult, however, to justify choosing the more complicated hypothesis until the consequences of the simpler one had been explored.

An hypothesis is nothing more than a scientific guess—it must never be accepted as a law until thorough tests warrant it. Some years ago, someone advanced the hypothesis that drinking milk was a cause of cancer. What was the observation that prompted this? It was that cancer was very rare in the early American Indian—who did not drink milk! An alternative hypothesis, namely that the Indian ordinarily did not live to the age where cancer is common, seems more plausible.

No hypothesis, no matter how extensively tested, can be accepted as having been proven. Future generations may—probably will—discard most of the important ones of today. As an example, for many years the principles of the conservation of energy and the conservation of matter were regarded as ultimate truths. Einstein's work made it necessary to fuse these two "laws of conservation" into one, since he showed that matter and energy are interconvertible. This hasn't changed things much in the ordinary laboratory, but it was, after all, the discovery that led to the atomic bomb and to the atom as a source of power.

Models—One of the tricks of the scientist is to use models. For example, the biochemist often isolates enzyme systems from cells and proceeds to study them in test tubes. In most cases, he hopes thereby to gain some insight into how they must function in the living cell. He would, of course, prefer to study them in the living cell itself, but usually this is impossible because many other enzymes are present, and many chemical reactions are going on simultaneously.

Another example is the use of a cotton pellet inserted under the skin of a rat to serve as a model for an actual rheumatoid arthritic lesion in the human. This pellet causes an inflammatory reaction, leading to an increased weight of the pellet as body fluids resulting from the inflammation diffuse into it. This increase in weight can be prevented or decreased by administering hydrocortisone or other similar steroids to the rat. The newer anti-inflammatory steroid drugs have been discovered by means of tests similar to this. Thus, the model has made it possible to find an agent active against rheumatoid arthritis, even though the test animal—the rat—is not subject to this disease.

Search for Causes—The most difficult thing about finding the cause of some natural phenomenon is setting up an hypothesis to be tested. Philosophers have pointed out certain clues that may indicate causes.

(1) *The method of agreement.* This method consists in observing that the various circumstances leading up to some event have one factor in common. This is a hint that the common factor *may* be a cause. The following story, however, proves that common factors are not always causes: A scientist who took too much Scotch and soda one evening had a nasty hangover next morning. The next time he went to a party, he changed to rye and soda, but he still had a hangover the following morning. On a third occasion he tried bourbon and soda—still he had the hangover. So he concluded that he would drink plain water with his whiskey in the future. Soda water, the factor in common on the three occasions, obviously caused the hangover! He might have reached a different conclusion had he remembered that there was another common factor—good old ethyl alcohol.

(2) *The method of difference.* The application of this method involves

the following assumption: If two sets of circumstances differ in only one factor, and the one containing the factor leads to the event, whereas the other does not, the factor can be considered to be the cause of the event. This method is important to our laboratories, because we use it in conducting chronic toxicity tests in animals. For example, we feed to four groups of rats identical diets and amounts of food. Three of the four groups, however, also receive graded amounts of the drug under study. If toxicity or death occurs in all the animals on the high dose of drug, but not in the other groups, we can conclude two things: (a) the drug is responsible for the toxicity, and (b) the drug is not toxic in the lower levels fed.

Of course, the above conclusions may not be valid; something unrelated to the drug *might* have caused the toxicity. This possibility is minimized by setting up the experiment with sufficient animals to reduce the probability of accidental toxicity to the vanishing point.

(3) *The principle of concomitant variation.* This principle states that if variation in the intensity of a factor results in a parallel variation of the effect, then the factor is a cause. Suppose for example, that when a new reagent is employed for the determination of blood sugar, the results are 10 per cent lower than when the older reagent is used. There are reasons to believe the new reagent may contain small amounts of iron. Is this iron the cause of the low values obtained? This question can be answered fairly conclusively by adding additional small amounts of iron to the new reagent and by repeating the tests. If the results are progressively lower as the amount of iron added increases, in all probability the iron was the cause of the difficulty.

Fallacies—The scientist must be on his guard constantly to avoid fallacies, most of which result from faulty reasoning.

(1) *Circular reasoning.* This means that the investigator accepts as proven a *part*, at least, of the thing he wants to prove. Suppose, for example, that one of you decides to prove that patients who have a fasting blood sugar level above 200 mg. per 100 ml. invariably have diabetes mellitus. To prove this, you determine the fasting blood sugar level of every known untreated diabetic who shows up. Sure enough—every one of them has a value higher than 200 mg. per 100 ml. Does this prove the point? Of course not, because the assumption has been made (perhaps unconsciously) that only diabetics have such high fasting blood sugar levels. At least the experiment was designed as though this were a proven fact. Of course, to do the investigation properly would require that fasting blood sugar determinations be done for every patient available, regardless of the diagnosis. If, having examined a large number of patients, *only* diabetics were found to have a fasting blood sugar level above 200 mg. per 100 ml., *then* you would be justified in formulating the *hypothesis* that this finding was sufficient for a diagnosis of diabetes mellitus. Remember, it still would be only an hypothesis, and not a fact (or law of medical science) until and unless it remained true even after millions of patients had been investigated. As a matter of fact, you know that diseases other than diabetes mellitus sometimes are accompanied by elevated fasting blood sugar levels.

(2) *Necessary and sufficient conditions.* Sometimes it is difficult to distinguish between conditions that are *sufficient* to prove a point, and those

that are *necessary* but not sufficient. For example, the experiment just discussed might yield data that would be *sufficient* to draw the conclusion that elevated blood sugar levels are common in patients with diabetes mellitus. These same data would be *necessary* to support the broader statement that only diabetics had elevated fasting blood sugar levels, but obviously, if not supported by more observations, would not be *sufficient*.

(3) *The troublesome third factor.* Often experiments are set up in such a way that important factors are overlooked. Suppose a group of patients with what appear to be colds are given injections of penicillin. Since the doctor wishes to minimize the chance that some of them may be sensitive to penicillin, he also administers an antihistamine. Behold! Most of the patients are much better next day—and the doctor concludes that the penicillin is responsible for this. It has not occurred to him that this was the hay fever season—that most of the patients had hay fever rather than an infection—and that the antihistimine—*not* the penicillin—was responsible for the clinical improvement!

(4) *Chance effects.* Another common error is to draw a conclusion from too few facts. Suppose we measure the growth-promoting effect of a diet using only one rat for the experiment. Obviously, the result obtained may differ completely from the average obtained if we had used twenty animals. For example, by chance this lone rat might have had an unnoticed infection that profoundly decreased the growth rate.

(5) *Bias.* This is an extremely common cause of error, especially in clinical research. If the doctor firmly believes in the effectiveness of the medication, and makes this evident to the patient, often the patient responds in a favorable way. This is the reason many clinical experiments are run as "double blind" investigations, in which the experimental drug and a placebo of identical appearance are given to patients on a random basis. Neither doctor nor patient should know which agent was administered until after the experimental results are evaluated and the "code" is broken.

Sometimes the patient—not the doctor—is biased, usually because of confidence in the doctor. I was once a pharmacist, and I remember an occasion when a worried wife called me about some capsules prescribed by her husband's physician. The husband had been in an automobile accident, and was in what seemed to be intense pain. When his wife gave him one of the white capsules, however, the pain disappeared and he went to sleep. His wife wondered if the medication were not "too strong." I assured her it was not. I did not tell her that each capsule contained 300 mg. of common baking soda!

(6) *Unknown variables.* Even today, experimental results that seemed to be firmly established are being questioned because variables previously unknown or unrecognized have come to light. For example, the vitamin requirements of the laboratory rat were worked out in literally hundreds of laboratories a number of years ago. Only recently has it been realized that as much as one-half the calories ingested by the rat comes from eating his own feces. When a device that prevents this coprophagy (eating of feces) is used, the vitamin requirements have been found to differ significantly from those accepted in the past.

B. Some Practical Considerations

Introduction—The first important question to ask when you are designing an experiment is—"What is the aim of this experiment?" All too often

at the conclusion of an experiment the facts revealed are not sufficient to answer the objective sought. Obviously, too, the experimenter should be thoroughly familiar with the work done in the field by other scientists. This will allow him to set up an hypothesis, however imperfect, that will help greatly in designing a meaningful experiment.

Oftentimes, the wisest approach is to set up a simplified model experiment. This one experiment often does not give the final answer sought, but it may point out a promising way to obtain the desired answer. Suppose we are suspicious of the chronic toxicity of a new active compound. We may feed the compound to a few rats for a month or so at one or two levels in the diet. If the rats thrive on it, probably it would be in order to set up a more elaborate study, involving many more animals, more levels of drug in the diet, and a much longer period of time. On the other hand, if all the rats die even though each receives daily only the amount of drug required to demonstrate biologic activity, the more complicated study would be unnecessary—because obviously we would not have a useful drug!

Variables—As has been pointed out, scientists must work with the assumption that similar results will be obtained if the circumstances of two experiments are closely similar. In other words, a second experiment will verify the first one if the *variables* are the same in both cases. Unfortunately, often some of the variables are unknown or if known, are uncontrollable. Scientists try to be sure to recognize and to control variables when this can be done. If uncontrollable variables are involved, the results of the experiments must be recognized as true "under the conditions of the experiment." Thus, conclusions drawn from the data obtained in experiments almost never can be said to be certainly correct. This explains why so many competent scientists disagree violently with each other's scientific conclusions.

Controls and Standards—Since variables cannot be controlled completely, it often happens that a somewhat different result will be obtained if an experiment is repeated. Fortunately, in most cases, this does not matter too much, because usually the answer sought is not in itself an absolute one. It may be a comparison with some standard—e.g., do patients respond best to drug A or to drug B? If this is the case, one of the two drugs can be thought of as a standard to which the other can be compared. If the experiment involves certain well known serum chemistry measurements, Versatol* is an excellent standard that will minimize the variables in the serum! If we wish to measure the activity of a new drug said to lower blood pressure, obviously we should control the experiment by giving some patients the active drug and some a placebo. In some cases, a single patient can serve as his own control—i.e., we may give him the drug for a week, followed by administration of the placebo for a week, and so on.

Choice of Sample—The choice of a proper experimental sample is very important. If we wish to find out whether vitamin B_{12} is required by human infants, we should not select obviously healthy infants ingesting diets known to contain plenty of vitamin B_{12} as our experimental subjects. Rather, we should search for malnourished children who are not

* Versatol is Warner-Chilcott's trade mark for its normal blood chemistry standard and control.

receiving any, or at least not adequate amounts of, food of animal origin. (Vitamin B_{12} occurs in animal foods.) If we wish to establish the standard level of hemoglobin in a certain locality, obviously it would be wrong to measure the level of this protein in the blood of sick patients in the hospital, ignoring the healthy people in the community.

Replication—It cannot be emphasized too strongly that important experiments should be repeated until reasonably constant results are obtained. Our laboratory data books contain many examples of "famous first" experiments! I'm sure that many of you do your analyses in duplicate, and repeat determinations when you obtain values that are abnormal. Human error and unknown variables cannot be minimized in any other way.

Level of Significance—All experiments involve the possibility of chance. Crucial laboratory and clinical experiments should be set up with this possibility in mind. Often this is done best by designing the experiment with the aid of a biostatistician. His calculations will make it possible to determine the probability that the result obtained could be due to chance rather than to the variable under study. In many biological and clinical experiments, the scientists attempt to utilize conditions and numbers of subjects so that the result will have a level of significance of 5 per cent or less. This means that, on the average, if the experiment were repeated 100 times, *and if the results were due to chance alone,* 5 (or fewer) times out of the 100 results identical with those observed in the first experiment would be obtained.

C. A Case History

Perhaps the best way to illustrate how scientists go about their daily work is to tell you the story of the discovery and development of a drug. The drug is Nardil,† a relatively new agent that is effective in treating mental depression. The first step involved collecting and evaluating certain scientific observations. The following things were known, because they had been published in scientific journals by other scientists:

(1) When reserpine is injected into mice, the animals are profoundly depressed for long periods of time.

(2) When reserpine is injected into mice, the brain cells of the animals lose their ability to store, but not to synthesize, two substances known as serotonin and norespinephrine.

An hypothesis involving the activity of serotonin and norepinephrine in the brain had been published. It was that serotonin acted in such a way as to cause sedation, whereas norepinephrine acted in such a way as to cause mental stimulation. Obviously, if this hypothesis is true, when reserpine is injected into mice, the resultant activity observed indicates that serotonin is present in excess—the stimulant effect of the norepinephrine is swamped out by the greater depressant effect of the serotonin.

At this point, a new hypothesis seemed to be indicated. Serotonin and norepinephrine are known to be inactivated in the body by an enzyme, monoamine oxidase, present in many tissues of the body, including the brain. Presumably, then, when these two substances are released from the brain cells of an animal that has received reserpine

† Nardil is Warner-Chilcott's trademark for phenelzine dihydrogen sulfate.

they remain active only a short time—until they are destroyed by the enzyme. The new hypothesis was this: If, before the reserpine is injected, the monoamine oxidase present in the brain is inhibited or destroyed, reserpine might cause the animals to react in some new way. Now, this may strike you as being a rather incomplete hypothesis—it seems to say only that "something will happen." This is true—but then the majority of the hypotheses used by the ordinary scientist in the laboratory are like this!

Fortunately, another experiment done in 1952 for an entirely different reason indicated that a drug known as iproniazid was an effective inhibitor of monoamine oxidase. Accordingly, in order to test the hypothesis, mice were given this drug (used at the time in the therapy of T. B.). If these treated mice were given reserpine several hours later, instead of being depressed, they became hyperactive. This state of over-activity lasted for days—just as had the state of depression observed when reserpine alone was injected. In other words, under these experimental conditions, the mice behaved as if they had an overdose of norepinephrine in their brains.

Now, reserpine is used in the treatment of excitable and mentally ill patients, and perhaps it is not too surprising that one of our scientists came up with a deduction. It was this: This drug, iproniazid, and perhaps other as yet unknown related ones, should be useful in treating patients who are depressed. It was not apparent whether reserpine also would be necessary *if the patient already were depressed.*

This deduction was tested in the clinic, and sure enough, iproniazid was found to be very useful in treating depressed patients. It turned out that reserpine was not necessary for this activity.

Unfortunately, it soon developed that the doses of ironiazid required to obtain the favorable results seemed to cause serious toxicity in some patients. This led to another hypothesis. It was that chemicals having the antidepressant effect of iproniazid, but without its serious toxicity, could be found. Accordingly, our chemists made a number of substances having some of the structural features of iproniazid. These substances were tested by our biologists. After a time, examination of the accumulated data indicated that one of these, now known as Nardil, was worthy of study as a potential drug.

At this point, a number of scientists in several laboratories had to begin to accumulate experimental data and to set up hypotheses and deductions. Some of the problems to be solved were:

1. What type of biological reaction did Nardil cause in the brain?

2. Was Nardil sufficiently free of toxicity to justify giving it to large numbers of human patients?

3. Since Nardil had a bad taste and was somewhat unstable, could a way be found to make a suitable dosage form—i.e., tablets (as it turned out)?

4. Could we devise a method of manufacturing Nardil on a large scale at a price that was reasonable?

When all of these experimental investigations had been carried out, we found it possible to make two highly satisfactory deduction or predictions:

(1) Nardil would be a relatively safe drug.

(2) Nardil would be effective in the treatment of mental depression.

As you know, or will have guessed, the clinical trials verified these predictions, and Nardil is widely used by doctors today.

It is only fair to point out to you, however, that useful results often are found even if the hypothesis used in setting up the experiments was in error. This may be so in the story of Nardil. For example, when Nardil is injected into a mouse, the monoamine oxidase activity in its brain disappears in a few minutes. It requires two or three weeks for the animal to make a new supply equal to that he possessed before the drug was injected. We might predict, then, that a single dose given to a human patient would produce a similar immediate and long-lasting effect. Actually, Nardil is given three times daily for from three days to three weeks or so before a beneficial effect is observed. True enough, when an optimal clinical result is obtained, the dose is reduced—but often it is necessary to continue giving the drug for months before it can be discontinued. (Fortunately, most non-psychotic depressed patients recover.) Moreover, in the mouse it is necessary to follow the administration of Nardil by the administration of reserpine in order to observe the obvious mental stimulation. This is not necessary in the human. Does Nardil act in the human by inhibiting the monoamine oxidase present in the human brain? We don't know! We do know that Nardil was discovered by observing that it did inhibit the monoamine oxidase activity in the brains of mice!

D. In Summary

If, then, by scientific method we mean the way in which scientists go about their daily work, it consists of the following four steps:

1. A study of experimental data collected either in the library or in the laboratory.

2. Choosing certain of these data that seem to the scientist to be related to each other. The ability to make a proper choice is the hall mark of the competent scientist.

3. On the basis of these facts, setting up a scientific guess, or hypothesis, that can serve as a springboard for making deductions or predictions as to the outcome of future experiments. The ability to formulate significant hypotheses has been the hall mark of the scientific genius.

4. Finally, testing the deduction, and hence the hypothesis leading to it, by experimentation.

I am sure that these methods used by scientists will not seem new or strange to you—for, after all, are you yourselves not scientists? And did not you use the methods of the scientist in your laboratory only last week?

OPPORTUNITIES FOR APPLICATION OF THE SCIENTIFIC METHOD IN THE ROUTINE CLINICAL LABORATORY*

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The function of the clinical laboratory is to provide the physician with tests and analyses that will aid in the identification and interpretation of disease states. It is the obligation of the laboratory to provide the best tests available, not necessarily the newest or the oldest. Therefore, it behooves the clinical chemist to be able to evaluate new tests with respect to accuracy, precision and clinical usefulness, and to be able to recommend their adoption or rejection.

The scientific method, as it applies to the clinical laboratory, would demand the following of the individual: (1) a knowledge of the underlying chemical and biological principles of an analysis; (2) an awareness of the variables which may be encountered in an analysis that could affect the results; and (3) an ability to devise methods of evaluating variations in methodology and determining the superiority of one test over another.

The opportunities for research in the laboratory derive from the availability of clinical material and professional assistance in correlating the results of clinical tests with pathology. This research would primarily fall into the following categories: (1) improvement of existing tests; (2) the evaluation of new tests as they appear in the literature; (3) the invention of new tests; and (4) the discovery of new applications for existing tests. Probably most clinical laboratories would be concerned only with the first two of these. To illustrate the type of experimentation which can be done in the clinical laboratory, I will present some experiments done in my own laboratory. I will limit the presentation to experiments with serum enzyme determinations, because of their current importance and unique nature.

In interpreting the results of an enzymatic analysis, it must be borne in mind that the analysis is a measure of a biological activity, not a substance. The answer is not necessarily a measure of the amount of enzyme molecules present but is a measure of how much work these molecules can do under the restrictions we have imposed on them. These restrictions are in the form of incubation time, temperature, pH, substrate concentration, etc. Changing any of these conditions will alter the enzymatic activity.

Lipase, an enzyme which is elevated in the serum of patients with pancreatitis, presents some rather unusual problems for the analyst. In the first place, the substrate changes in composition during the enzymatic incubation as the triglycerides are hydrolized to diglycerides and then monoglycerides. Secondly, the pH changes during the incubation due to the liberation of free fatty acids. To counteract this pH change, the system is buffered, but since titration of the liberated fatty acids with standard alakali is the measure of lipase activity, this very buffering serves to preclude a sharp end point. Thirdly, a two phase system

* Read before the Workshop on Research and the Scientific Method in Medical Technology, 28th Annual Convention of ASMT, Atlantic City, New Jersey, June 1960.

is involved in which the substrate is in the oil phase and the enzyme is in the water phase. In the method of Cherry and Crandall[5], the substrate is an emulsion of olive oil which is not stable. The emulsion breaks down during the enzymatic incubation, thereby changing the surface area on which the water soluble enzyme can act. These three factors serve to complicate the kinetics of the reaction. Most enzymatic reactions are zero order reactions, i.e., the amount of substrate broken down is a linear function of time, at least until 5 to 10% of the substrate is used up. The relationship between incubation time and amount of lipid hydrolysis by the Cherry and Crandall lipase assay is shown in Figure 1.

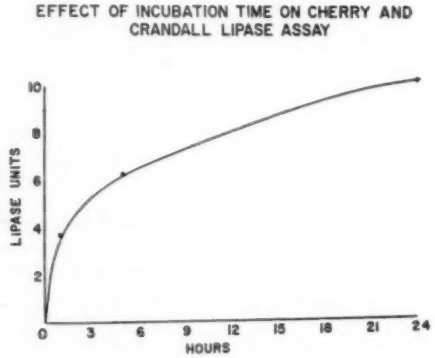

Figure 1

The effect of incubation time on the lipase activity of a serum sample containing added pancreatic lipase. Lipase activity is expressed as ml. of 0.05 N NaOH for each incubation time.

It is apparent that this reaction is not zero order for even a fraction of the 24 hour incubation time. More than ⅓ of the hydrolysis occurred during the first hour. The activity of various concentrations of pancreatin is shown in Figure 2. This also illustrates the marked deviation from zero order of this enzymatic reaction. When lipase units were plotted against enzyme concentration, a straight line was found on a semi-logarithmic plot between 0.1 and 10 mg. pancreatin/ml. This means that the difference in activity between 0.1 and 1.0 mg/ml. is the same as between 1 and 10 mg/ml. Thus the Cherry and Crandall unit is an erroneous measure of lipase concentration. In our own research, therefore, we use instead of Cherry and Crandall units what we call "pancreatin equivalents" which are calculated by reference to the curve shown in Figure 2.

In 1948 Boissonnas[3] published a lipase assay using as a substrate Tween 20, a non-ionic surface acting agent which is a water soluble ester of lauric acid. The water soluble characteristics of this substrate would presumably obviate many of the difficulties encountered with emulsified substrates. Indeed, when we assayed a dilution curve of pancreatin with this method, the activity was found to be a straight

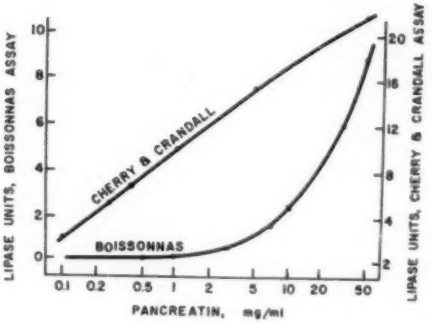
LIPASE UNITS, BOISSONNAS ASSAY
LIPASE UNITS, CHERRY & CRANDALL ASSAY
CHERRY & CRANDALL
BOISSONNAS
PANCREATIN, mg/ml
0.1 0.2 0.5 1 2 5 10 20 50

Figure 2
The lipase activity of pancreatin (3 x U.S.P., Wilson & Co.) in serum by the Cherry and Crandall and Boissonnas assay methods. The activity of the serum alone was subtracted from the total in each case.

line function of the concentration. This is shown in Figure 2 as a exponentially-shaped curve on the semi-logarithmic plot. It is apparent from the curve that this substrate is quite insensitive to lower concentrations of pancreatin as compared with the Cherry and Crandall assay. On the other hand, it was noted that normal serum with less than 1 Cherry and Crandall unit per ml. gave quite large titrations with the Boissonnas assay. In an attempt to explain this discrepancy, we followed a lipase purification as outlined by Boissonnas[4] with both assay methods. The data expressed in pancreatin equivalents per mg. nitrogen are shown in Table I. The pancreatin starting material had, of course, the same activity by both methods in pancreatin equivalents. The final solution gave a nine-fold purification by the Boissonnas assay, a figure which was in very close agreement with data of Boissonnas. On the other hand, when the activity was followed by the Cherry and Crandall assay, it was found to have been decreased by a factor of four during the purification. Thus it is apparent that these two assay methods do not measure the same type of activity. Probably the Boissonnas assay measures a non-specific esterase and not pancreatic lipase, and therefore would be of no value in the diagnosis of pancreatitis.

TABLE I
THE LIPASE ACTIVITY OF VARIOUS FRACTIONS OF A LIPASE PURIFICATION
Activity in Pancreatic Equivalents/mg. N

Fraction	Boissonnas	Cherry & Crandall
Pancreatin	10.8	10.8
Initial Extract	14.9	9.8
Final Solution	91.9	2.8

With respect to acid and alkaline phosphatase, pH is probably the most important parameter to control and for different reasons for the two enzymes. Serum alkaline phosphatase is an enzyme or enzymes

which is quite stable over a wide pH range, but the activity of which is markedly effected by changes in pH. This enzyme has a very sharp pH optimum at about 9.6. Serum acid phosphatase, on the other hand, and this would include both prostatic and erythrocytic acid phosphatase, has a broad pH optimum around 5 but is destroyed very rapidly at alkaline pH's. The effect of serum pH on acid phosphatase activity is illustrated in Figure 3. In this experiment, pooled serum to which had been added prostatic acid phosphatase was adjusted to various pH's and held at 25° C. At intervals the samples were assayed for acid phosphatase by the method of Shinowara, et al.[9] It is seen from the curves that as the pH of the serum was elevated above 7.8 the acid phosphatase became more and more unstable. Indeed, at pH 8.46 only 30% of the initial activity remained after 2 hours. Thus it is extremely important in acid phosphatase analyses to maintain the serum at a relatively low pH.

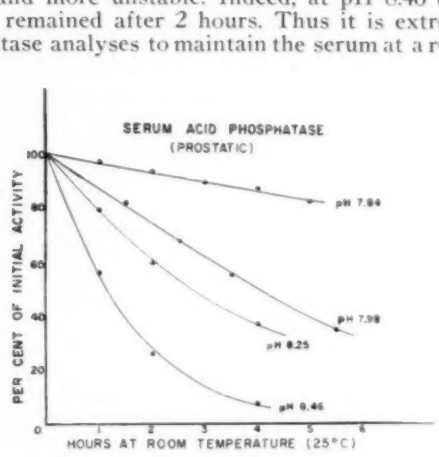

Figure 3
The effect of serum pH on the stability of prostatic acid phosphatase.

The pH of serum after it is drawn tends to go up due to the loss of carbon dioxide. Freshly drawn serum has a pH of around 7.6. If unprotected it will go up quite rapidly during the first few hours and reach a pH of about 8.5 or more overnight. The simple expedient of storing serum in small stoppered tubes rather than in large unstoppered tubes will prevent much of this pH change. This is shown in Table II. In this

TABLE II
THE EFFECT OF OVERNIGHT STORAGE CONDITIONS ON SERUM pH

Tube Size mm.	cc. of Air Above Serum	pH of Serum in Stoppered Tube	pH of Serum in Open Tube
10 x 75	1.5	8.04	8.26
13 x 100	7	8.12	8.34
18 x 150	25	8.21	8.53

experiment we added 2 ml. of pooled serum with a pH of 7.98 to 3 sizes of test tubes, half of which were stoppered and half of which were left open. After standing overnight in the refrigerator, the pH of the various sera were measured. In the small stoppered tube the pH was practically unchanged, while in the larger open tube it had increased over 0.5 pH units. Another method we have found useful is to add disodium citrate to the freshly drawn serum at about 10 mg/ml. This immediately lowers the pH of the serum to about 6.5 at which point the acid phosphatase is quite stable.

The effect of serum pH on alkaline phosphatase activity is shown in Figure 4. In this experiment a serum with elevated alkaline phosphatase was adjusted to several pH's between 7.50 and 10. We purposely used pH's that were above the physiological range such that we would be sure to get points on both sides of the pH optimum of the enzyme. The adjusted sera were assayed for alkaline phosphatase activity by a modification[6] of the Bodansky[2] procedure and the quantitative Phosphatab* method of Klein, et al.[8] The Phosphatab activity is in terms of Bodansky units for comparison. Two facts are apparent. First, the veronal buffer of Bodansky at pH 8.6 is at the wrong pH for optimum enzyme activity. Secondly, this buffer is too weak to counteract changes in the serum pH and thus maintain the pH of the reaction mixture at a constant level. In the physiological range of serum pH, the method of Klein, et al. showed a variation in activity of only 7% while a variation of 47% was obtained with the Bodansky method. This experiment was repeated limiting the pH to the physiological range but extending the experiment to include the assays of King and Armstrong,[7] Shinowara, Jones and

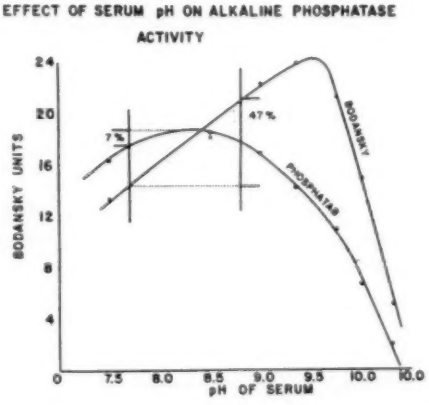

Figure 4

The effect of serum pH on alkaline phosphatase activity. The vertical lines denote the physiological range of serum pH.

* Available as PHOSPHATABS (alkaline)—patent pending—from General Diagnostics Division, Warner-Chilcott Laboratories, Morris Plains, N. J.

Reinhart[9] and Bessey, Lowry and Brock.[1] These assay methods are all different with respect to the pH and buffering capacity of the buffer. The data are shown in Table III. The Bodansky assay, greatest affected by the serum pH, uses a weak veronal buffer at too low a pH. The assay of Shinowara, et al. uses the same weak veronal buffer of the Bodansky but at a higher pH. Thus the buffering capacity is not increased but the pH is more optimum, resulting in less variability. The King-Armstrong assay employs a stronger veronal buffer, the pH of which again is below the optimum for the enzyme. The assay of Bessey, Lowry and Brock uses a glycine buffer which is quite strong at the pH used. The decrease in activity noted with increase in serum pH results from the fact that the pH of this substrate is around 10.3 which is higher than the optimum for the enzyme. PHOSPHATABS (alkaline) employs a strong tris buffer designed to give a reaction mixture pH with serum of 9.6, and the assay of Klein, et al. was found to give the least variability in activity with variation in serum pH.

TABLE III
EFFECTS OF SERUM pH ON ALKALINE PHOSPHATASE ASSAYS

Method	pH 7.6	pH 8.2	pH 8.8	Change
King-Armstrong	18.3	20.2	22.5	23%
Bodansky	6.9	8.3	9.6	40%
Shinowara, et al.	9.6	11.8	12.1	26%
Bessey, et. al.	6.4	6.0	5.8	—10%
Klein, et al.	8.2	8.2	8.0	— 2%

These three examples should serve to illustrate the application of the scientific method to a clinical analysis: a knowledge of the underlying chemical and biological principles, an awareness of the variables which may affect an analysis and the ability to devise methods of evaluating variations in methodology. Always be on the lookout for new methods and new ways to improve old methods. But be wary of adopting a new method routinely until you are convinced of its validity. To paraphrase the advise of Polonius to Laertes, "*Be* the first by whom the new is tried *and* yet the last to cast the old aside."

Acknowledgment

The valuable technical assistance of Miss Sylvia Malament for the lipase experiments and Miss Prunella A. Read for the phosphatase experiments is gratefully acknowledged.

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PROBLEMS IN VALIDATING RESEARCH*

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May I preface my remarks by saying that each of us, on a very daily basis, validates the results of research through the procedures run in the clinical laboratory. Most of the procedures are valid. Valid because the data obtained through each determination is that which we anticipate as a result of the original publication—or to put it another way—we know the test will work each time it is run.

At the same time, as Dr. Arnow has mentioned earlier, "Some *hypotheses* have persisted so long and seem so obvious to us that we are willing to call them laws of nature, and in fact, are willing to believe in their absolute truth." This same feeling, I am sure you will agree, persists in many laboratories. Some methods and techniques still enjoying very wide use were developed around the turn of the century or shortly thereafter. The remark is not too infrequently heard—that this is the only way to do it—or—we have been doing it this way for such a long time it would take complete re-training or everyone would be confused.

Many of these old techniques have satisfied a need—but can we, as professionals, rest upon our oars without attempting a break-through to more accurate and useful knowledge—and at the other extreme, should we accept every new test or technique that comes down the pike just because it is new? Obviously not. Somewhere there is a middle ground where the laboratory drops something old when it can be replaced by something that can be more useful.

Each of us, as laboratory workers, has a real interest in research. Research, I would like to define, as the use of prior knowledge, reason, and imagination to create a hypothesis. The evolution of this hypothesis to theory, upon which can be built the cornerstone of experimental method through which, by diligent application of effort, one can reach a logical and proveable conclusion. Academically and technologically, we are becoming better equipped to take this direction as each day goes by. Let us explore the avenues open to us. The progress, the evolution, the very existence of the clinical laboratory, depends upon all forms of research—pure research into the basic sciences as well as development and clinical research.

Our area, I'm sure you will agree, takes us into the area of developmental and clinical research. The area that creates new methods and procedures leading to better laboratory practice, giving the clinician better information to pin-point patient disorders and help in correcting them. How are these methods and procedures evaluated and validated so that continuing development can ensue? Francis Bacon, one of the fathers of modern scientific thought has stated, "The scientific man has in fact to practice two mental processes, the making of a discovery and the demonstration of its truth. Essentially, the two processes are distinct and the one might be largely developed while the other is in a state of relative arrest."

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The demonstration of the truth mentioned by Bacon is required to prove the validity of research. The demonstration of proof is perhaps one of the most important systems of scientific checks and balances. Only by applying these checks and balances to each other—objectively—can the truth, the validity of research be distilled by us. How do we go about this? When a discovery is made, from experiment right through to conclusion, we apply for proof. Proof from our colleagues, hoping they will validate the work. A communication is prepared and we publish.

The structure of the study usually follows a classical form. Through the use of prior knowledge, a hypothesis is formulated and an attempt is made to prove or disprove it. Experiments are performed, the data organized, and eventually a communication is prepared. It is through this communication that we let the reader know—the purpose of the work, the manner in which it was performed, the data that was obtained, and, finally, an interpretation of the results—that is, what do they mean? The format of most scientific communications is generally the same. They usually open with an introduction to what has preceded, and an indication of a need. There should also appear a clear and explicit statement of the purpose of the work. Why was the study undertaken—what is it intended to show? The next section is by far the most important, for it is here that the methods are defined. The methods by which the results were obtained. Very often we have a tendency of making many assumptions, the most dangerous one is—that other investigators have the same background and knowledge and will be perfectly capable of duplicating the results without the need of outlined specifics. We must not assume. A great deal of care and detail should be devoted to the presentation of methods and materials, for this is the point that can be most important in validating the results of research. In this section of the communication, specific and detailed instructions should be included for the performance of each phase. Such information as the source of purity or grade of the chemicals used; have other lots from the same source been used; has the same chemical from other sources been used? At times, such things as temperature, humidity, barometric pressure can be important factors. Complete records should be kept during the experimental period. It is just as important to indicate in the communication, those conditions which do not affect the outcome of the experiment as it is to define those that do.

The next section of the communication should deal with the data obtained by the use of the methods and the materials outlined. The data should be presented in a clear and concise manner. This can take many forms. You all know and have used these forms. They can include simple tables—charts—bar graphs—distribution curves—or a combination of several that will give the clearest possible presentation. When applicable, a statistical analysis should be done. When such analyses are performed, the specific formulae used should be given.

The next sequence deals with the conclusions drawn as a result of the experimental data. The inclusion of subjective or emotional bias or any other fallacy of faulty reasoning should be assiduously avoided. This is then the map, too, for all future travelers down the trail of this

experimentation. Like the explorer, the original investigator must blaze his trail accurately—objectively—so that others may follow and the future may be built.

Once having accomplished an objective piece of work, and it has been communicated through a publication, it becomes time to demonstrate its truth. Truth in the sense, can other investigators duplicate the data. Before attempting to duplicate the work, the validating investigator should clearly understand the purpose of the original study. What was it intended to show? With this clearly in mind, the validating investigator can examine the methods and materials. Is their source, grade, purity, availability, clearly outlined? If some have to be prepared in the laboratory, is there a method for their preparation and a technique to determine proper preparation included? Are the specific conditions under which the experiments were carried out clearly defined? Is the data clearly presented and understandable? Are there facilities and equipment as specified in the original communication available? In attempting to duplicate, to validate the results of another investigtor, the methods, materials, and techniques should be exactly the same as those defined in the publication by the original investigator. The data should of necessity bear out the conclusions for which they are the basis. All too often we find the introduction, the purpose of the work, the results and summary read, but the data taken for granted or completely ignored. When an experiment is performed by two different individuals using identical methods, reagents, and techniques, they should get the same data. Anything beyond this may be open to discussion and disagreement.

When individuals of different backgrounds and training objectively look at the same data, they examine it in the light of their own knowledge and experience. They may come up with entirely different impressions and interpretations. These may be due to many reasons, on of which may be a difference of opinion on the original hypothesis. Another may be due to fallacies having been introduced by faulty reasoning. When this occurs, and it can be established that one of the many variables that must be accounted for in the experiment has not been controlled or evaluated, the entire experiment must be re-evaluated in the light of the new information. The data that is then obtained may be used for the conclusions and substantiation of the hypothesis.

In advocating the validation of research, I do not mean to imply any dishonesty or irresponsibility on the part of the investigator. As mentioned earlier, it is a system of checks and balances. All too often we hear of a technique that will work in one laboratory and will not work in another. The attempt is being made to see that all techniques work in all laboratories. There is an ever-growing interest in this field. Some professional organizations have already taken an active part in it. One group of professional chemists has set up the mechanics for this type of work. When an author submits a new technique for consideration and adoption, it is evaluated independently and objectively along with the current used technique. Each validating laboratory sends in its report and recommendation. These reports are then evaluated and a final recommendation made. Should the newly-submitted technique offer advantages over what has been available in

the past, a recommendation is made to adopt the new test as the official test for that particular determination. This is in no way meant to discourage research into new techniques. I believe you will agree with me that it will discourage the presentation of inferior techniques or techniques that are only just as good. I'm sure you'll agree with me it will stimulate research into even better methods, for the better method will become the official method, but only until a still better method is found.

Still another group of professional chemists has approached the problem in another way. During the past few years published clinical laboratory methods have been examined. They have been submitted for consideration by a responsible member and have been checked in other laboratories. These methods have then been complied into published volumes. They may not be the best available method. However, if performed as indicated in the publications, the laboratory can be assured of getting reliable results.

Some of the European publications have approached this problem in a different and unique manner. When a paper is published in the journal, and another investigator takes issue with what has been presented, he may write a letter to the editor, stating his position and requesting an explanation or clarification. This letter is published in the journal and it becomes part of the literature. The original author may then reply again as a letter to the editor, which is published. The reader has before him both sides of the controversy. We have been following such a series for the past eight months, and have been watching both sides of the argument. This discussion in print has concerned only the validity of the thinking used to draw conclusions in the original publication.

We here in the United States, to the best of my knowledge, do not have such an opportunity. The best we can possibly hope for when we disagree with a publication, is to publish again. There then are many publications to follow, and it becomes difficult to follow a controversy. From an examination of the literature it would appear that it is a great deal easier for a validating investigator to publish the results of his work when they confirm the original publication, then it is for a validating investigator to publish work that disagrees with another.

We in the General Diagnostics Division are extremely conscious of the responsibility and the need for valid research. For this reason a department, a Department of Clinical Investigation, has been established whose sole responsibility is that of validating research. To the best of my knowledge this is the only department, as such, specializing in laboratory methods, that exists in the organization of the many manufacturers and suppliers to the clinical laboratory. The responsibility we feel is so great, that even though our own research facilities may be at least as good as the best, we demand that every new reagent or technique that is evolved from these laboratories be subjected to the rigors of validating investigators and research. Months, and usually years, are spent in this type of work prior to general availability of a new technique. In almost every instance, a cross section of the laboratory population works in this validating program. When finally a publication is presented, and a new tool for the laboratory is made available, it should never be said *generally*: "It works in your lab but not in mine!" It has worked in labs just like yours and it can work in yours. This type of validating research provides another important function. Often in the process of this research,

a distant laboratory may be faced with a problem that may not have been encountered before. Several such instances have occured. I would like to tell you about a few just to illustrate the points I have made.

Recently, in a study that was designed to determine the most effective method for the control of anticoagulant therapy, a most interesting phenomenon was observed. One of the reagents required was barium-sulphate adsorbed plasma. In an attempt to keep the system as constant as possible and have the highest purity of reagents as one could get, the plasma was adsorbed several times with barium sulphate. When an attempt was made to check it, the plasma was found to have lost all its activity. Further study showed that only one adsorption on the barium sulphate gave the best activity. In this instance, a highly "purified" plasma, supposedly, was not as good as one "not quite so pure."—The most frequent problem encountered in validating research is just the opposite. The questionable purity of reagents makes validating difficult, as the following example will show.

A reagent was prepared which had a known concentration of cholesterol. This was sent to many validating investigators. Their recoveries were as varied as the various parts of the country where they worked. There was one consistency, however, in all their results. They were all consistently high. It was logically impossible to get more out than one puts in—so the start of a project was in sight. Many laboratories worked on the problem and the notes I shall make are a compilation of the variables from them all. All of the reagents used in the many cholesterol methods were carefully examined. The cholesterol—crystalline cholesterol used as standard—was examined both by infrared and by melting point determinations. The materials available had varying purities. The cholesterol finally used, had to be re-crystallized several times (four times out of 95% ethyl alcohol and finally once out of absolute alcohol) in the laboratory. Following this re-crystallization, the cholesterol than had the physical characteristics it should have had. These crystals had to be stored in a vacuum dessicator and new standards prepared at regular intervals. When a standard was prepared, its activity was recorded every day. When a new standard was prepared, it was compared to the old before being put into use. It was also learned that upon standing for 48 hours, these cholesterol standards lost about 15% of their activity. When a new standard was prepared, it was allowed to stand for 48 hours before being put into use. Following this original decrease, the activity was constant for 30 days. Using the various cholesterol techniques validating investigators also discovered that the reagents and reactions that are currently being used as well as some new ones that are being proposed, are not specific for cholesterol. They are affected by many compounds, including bilirubin—steroids—vitamins—as well as many other substances that are still not identified. Unless the technique uses some form of purification to remove some of the contaminants, erroneously high values will result. This purification can take the form of an extraction step or perhaps a precipitation step. During these studies it was learned that the purity of the glacial acetic acid used was extremely important. Upon standing and exposure to light, acetic acid can break down to glyoxal, which will react with tryptophane in serum to give apparently higher cholesterol values. After the elucidation of all these variables which

can influence the test, but which do not always occur, and by using techniques to control these variables, each of the validating investigators had no problem.

This then, perhaps illustrates the function of validating research and some of the problems that one encounters in such work. A concentrated effort should be made to see that a method or technique can work in every laboratory that follows the published procedure. The only way to know whether it will or not is to have other investigators take the published procedure and attempt to duplicate it—that is—to validate the research. It is extremely important to discern whether every bit of information necessary to perform the experiment has been presented. Research in its broadest aspect—in every field and every form—encompasses three facets. These are: experimentation, communication and understanding. We, through the Department of Clinical Investigation, hope we can stimulate you into further experimentation, clear and precise communication, which can lead to a better understanding of the professionals that you are.

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Continued Page 397

"PUBLIC HEALTH BENEFITS FROM INCREASED CLINICAL LABORATORY INQUIRY"*

LEONARD A. SCHEELE, M. D.

Senior Vice President, Warner-Lambert Pharmaceutical Company, Morris Plains, N. J.

Miss Gallaher, members of the American Society of Medical Technologists, friends—during your 1958 convention, I had the very great privilege of addressing your opening session. That year was, in many ways, a turning point for Medical Technology as a profession. It is an honor to be asked again—after the wonderful advances your profession has made since then—to point some directions which the research-minded members of the profession of Medical Technology might now look toward—directions whose first paths are available every day in your work as teachers, as students, and as practicing scientists—and directions which afford potential for major contributions by medical technologists to solution of public health problems in this country and throughout the world.

As an intimate of these public health problems and of your profession for many years, I hope today to stand in the role of a "matchmaker"—to attempt to bring together some of our vast unsolved problems in public health which cry for new approaches and increased attention—to bring these problems together with your own enthusiasm, brain power, and opportunity for contributions.

I understand that your annual banquet and presentation of awards will take place tomorrow night and that many awards will be presented for papers of outstanding merit. I understand, too, that the number of papers eligible for consideration for awards—and their quality—has been increasing considerably over the years so that the task of judging winners becomes increasingly difficult for your Awards Committee. I look forward to the day when another awards committee may present its award for research in medicine by a medical technologist or group of them. The committee which I would most like to make this award would be one like one of the Laskes groups, or let's shoot for the moon, the Nobel Group.

The last Nobel Prize in medicine for research in an area of vast public health significance was granted in 1954 to Weller, Robbins and Enders for their work on polio virus. And the award before that was in 1951 to Theiler for his work on yellow fever. I believe that it is of great significance to you that these studies, like many major public health studies before them, were for work performed in the laboratory, on material from patients. This work facilitated the control of diseases which were annually taking the lives of a great number of human beings. I believe it is significant, too, that these contributions were in the area of Microbiology and Infectious Diseases on which most public health attention was directed for half a century.

As a result of the contributions of these men and their predecessors and successors, infectious diseases have been lowered in rank so that

* Presented before the Workshop on Research and the Scientific Method in Medical Technology, 28th Annual Convention of ASMT, Atlantic City, New Jersey, June 1960.

they are no longer the leading causes of death in the United States today. Now, other diseases, which afflict a large part of our growing and aging population cause the greatest discomfort and unhappiness and account for many deaths. I refer, of course, to chronic and crippling illnesses. These problems are crying out for solution, as you know from your own experience.

The alert medical technologist is in an excellent position to detect unexpected evidence of abnormality—signs which usually are not intentionally looked for. You are in an excellent position to make such observations as you do your regular work. In the small community hospital there is this advantage—the medical technologist can often recognize, by personal contact, the patient re-admitted several years after a previous hospital study, with a chronic disease whose early warning may have been given in observations years before—observations not then thought to be significant.

I have said that the medical technologist of today and tomorrow is in an unsurpassed position to contribute to the advancement of public health. Now, I would like to go more deeply into today's areas of public health need and describe a few of the possibilities for research by medical technologists. Possibly a take-off point for research areas would be to take those gross areas which have been recognized by the United States Congress when it established the National Institutes of Health in Bethesda, Maryland. The gross areas, each centered in an Institute are: Allergy and Infectious Diseases; Heart Disease and related ills; Cancer; Arthritis and Metabolic Diseases; Dental problems; Neurology and Blindness; and Mental Health. On the surface, the names of some of these Institutes may sound far afield from the work of the medical technologist. But I have said I have come as a "matchmaker"—to point up some unsuspected interests that you may have or could develop in these areas.

Allergy and Infectious Diseases have been grouped together because today's scientists believe that they share a common base in antigen-antibody or immunological approaches. Hay fever, asthma, skin sensitivities are among the common ailments of man. Certainly, during the hay fever season, specimens drawn for routine serology are often taken from individuals with the characteristic sneezes, wheezes and runny noses caused by this common allergy problem. Despite the fact that the purpose of your serological testing may lie in study of a totally different problem, one might try to find serological tests and approaches to this and other allergy problems. These would be helpful in improving our understanding of these problems and in their prevention.

In another area closely related to this—only within the last decade have we come to recognize the frequent association of cerebral palsy in children with Rh incompatibility in the mother. This is a good example of how a laboratory procedure has aided medical knowledge.

Because routine serological and routine immunohematological testing are performed today on such a wide cross section of our total population—a development of the last decade or so—you have in your hands, the possibility of gaining valuable information on relationships between these determinations and the common diseases of mankind. An example

of a recent finding of this type is the association of the "A" Blood Type, more frequently than any other type, with peptic ulcer in males which may lead to new research approaches to this disease of civilization.

Each of these new observations—Rh incompatibility in the mother associated with some cases of Cerebral Palsy—a specific blood type being identified with peptic ulcer patients—serve as the basis for research and you may be able to open doors in other diseases by relating your careful observations with clinical reports.

The problems of heart disease and its allied conditions, kidney diseases, and vascular disturbances in general, represent one of today's most challenging areas of research. In dealing with any problem successfully, one must first define it and proceed, when possible, to clarify the cause. In heart disease, most causes, other than streptococcus infections, remain to be proven. The interest in increased blood cholesterol levels and their possible role in the development of atherosclerosis has been complicated by the inadequacy of methods for measuring cholesterol levels. The development of more accurate and precise methods of measurement, and of simpler methods, checked daily by adequate measures of control in the clinical laboratory, could be a major contribution to the understanding of cholesterol's role in cardiovascular disease.

Another major need is the development of specific tests to enable recognition of biochemical changes signalling future heart disease . . . so that adequate preventive measures may be instituted. Such tests may already exist among the professional tools you use daily, but they haven't been recognized and have never been applied to this specific problem. The development of this application might, one day, save many lives. You can help here.

In kidney disease, traditional screening methods, such as that for albumin in urine—although indispensable to modern medical practice—have provided little information on the variety of kidney problems which affect large population groups. Newer tools for measurement—flame photometry for electrolytes—Warner-Chilcott's recent development of a simple paper test for urea nitrogen—now permit finer characterization of biochemical differences in different types of kidney and electrolyte disturbances. Because of their very newness, the application of these and other "tools" on a large scale is just beginning. Reports on your observations of their use can be helpful.

The cancer problem is one of the most publicized of all. Here, causes remain to be defined. There is scientific disagreement over a question like the causal relationship of heavy cigarette smoking to lung cancer. Recently a great deal of attention is being focused on viruses as a cause.

There is need for more information, with elaborate biochemical studies in large patient series. We especially need tests for the early diagnosis of cancer. Such tests are badly needed, not only to indicate that cancer is present somewhere in the body, but also to specifically pinpoint the organ or tissue affected. It may then be possible to give local treatment after appropriate confirming diagnosis by a qualified pathologist. And, as in cardiovascular diseases, we need tests that will allow us to predict a tendency to malignancy years before it develops. Hopefully, in cancer, as in nutritional deficiencies, we may then be able to prevent the dis-

ease. There are untold opportunities for medical technologists to work on these problems and make major contributions.

Mental health is one of the problems that the technologist ordinarily sees as very far removed from his or her daily work. The dramatic effectiveness of tranquilizers and antidepressants—as Dr. Arnow illustrated in his talk this morning—are bringing medicine today away from reliance primarily on psychoanalytic study and treatment methods to a realization that physiological and biochemical disturbances play important roles in mental illness, and that chemotherapy offers therapeutic help to many patients. Mental disease, today, hospitalizes one in every eight of our citizens. Extensive laboratory facilities are just not available in our large mental institutions. Less than 50 percent of mental hospitals, over 500 beds, operate clinical laboratories today, and usually the laboratory tests they offer are limited. The application of many of the tests which those of you here today use routinely to mental patients in an effort to discover significant differences in the disturbed patients, as against normal individuals, is a wonderful challenge to the professional technologist.

The National Institute of Arthritis and Metabolic Diseases handles research into nutritional diseases and into diseases caused by basic metabolic defects and endocrine disturbances, among many others. Taking endocrine diseases alone—as an example, simplified methods of steroid testing and the application of steroid tests to wider population groups can provide valuable information needed to understand many diseases.

Dental diseases certainly sounds like problems far afield from your work. They are problems in which you do have a role to play. Let me point to some of the ways. The application of some of the important tests for bone formation to saliva may provide significant information on the environment of the health of the decaying tooth. For example, Phosphatabs are being employed in studies of the alkaline phosphatase content of saliva; preliminary observations show that this enzyme is present in normal saliva and may be reduced in individuals with a significant tendency to caries. As methods become faster and simpler, tests for calcium, phosphorous and for enzymes, in large numbers of individuals, may prove helpful. Tests for differences in the levels of these constituents where fluoridated water is available might also prove valuable.

Finally, the area of neurology and blindness, too, is available for the attention of the sympathetic, research-minded technologist. A significant discovery in this area related to your own work, and made within the last decade, was that premature infants frequently suffered detached retinas and blindness as a result of receiving oxygen in their incubators. The entire field of study of metabolism in infants and children and in adults too, especially that related to the processes of aging, will bring important scientific information. You should play a major role in obtaining much of that information.

Of course, one must stress the fact that the primary function of the routine laboratory is service to the sick. This function must never be interfered with. However, there should be time for research, too. The

relationship between routine laboratory service and the investigative work is well illustrated by the following quotation from the World Health Organization's Second Report of the Expert Committee on Health Laboratory Methods, devoted to hospital laboratory services throughout the world. Dr. George Zur Williams, Chief Pathologist at the N.I.H. Clinical Center, is Chairman of this Expert Committee. The report said:

"There is another group of countries in which entirely different needs exist. Here hospital laboratories and central and state public health services are well developed but their efficient application to problems of the community at large is hampered by the absence of effective voluntary cooperation or integration. This situation requires intensified education and orientation of the medical student and young physician in resident training with regard to public health problems, epidemiological techniques and industrial and preventive medicine. Pathologists must learn the importance of public health application of their knowledge and methods.

"Areas of public health effort in which the cooperation of the hospital laboratory will become increasingly important are chemical analyses related to patients exposed to industrial toxic hazards, water and air pollution, and nutritional epidemiology with reference to the study of geriatric conditions (atherosclerosis, etc.). Studies in cancer must be included. In the future, other equally important ways in which the hospital laboratory can contribute to the public health effort will surely emerge.

"In considering the need for collaboration, it must be mentioned that to obtain the most effective integration in the interests of human communities, and to protect the medical and scientific integrity of the control of public health activities, medical personnel must be free from political and other external influences and control, and must be appointed and assessed solely on the basis of their professional and personal qualifications.

"Furthermore, the pathologists directing hospital laboratories must not permit their interest in public health problems to interfere with their services to the sick, or vice versa."

There is still one public health problem we have not discussed today. This is another problem where our interests—yours, in Medical Technology, and mine in the pharmaceutical industry—have been mutual for many years. That problem is that of finding enough young people to work in our laboratories and in our health sciences. There is a form of research, different from that we have discussed so far, which I hope many of you are conducting daily. This research is in study of educational methods, designed to capture the interest, to motivate, and to inspire young people to join your profession. The methods that you have developed can be of value to our science teachers in secondary schools and in universities in presenting a representative sample of health science to their students in an exciting fashion. Papers on what you have accomplished would be valuable to the Journal of High School Science Teachers, to the Journal of Chemical Education, and many

others. I hope that all of you will work seriously at this research and education job.

In his introductory talk, Mr. Cohen quoted Harriet Boyd when he said: "No person is truly educated until they begin to inquire themselves and begin research in their field." This applies to you, and you will be fulfilling your mission more fully if you do research. In addition, you should be one more part of the job of an educated person. That is, you should educate others to enter medical technology and research, and then—you should teach them to be good medical technologists when they decide to join you in your profession.

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Continued Page 404

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CONTENTS

Volume 26 **No. 6**

NOVEMBER-DECEMBER 1960

PAGE

Studies in the Glutathione Stability Test and Its Use in a Clinical Laboratory.....333
by Patricia Ann Amos

An Evaluation of Some of the Factors which are Responsible for the Colonization of the Nursery Area with Staphylococcus Pyogenes.....338
by Silas Farmer and Paul N. Ladefoged

Hemagglutination in Hepatic Disease.....345
by W. Paul Havens, Jr. and Claire Tissari

Immunohematology of Blood Therapy.....351
by Mary F. Eichman

The Bodian Stain: Its Use and Method.....360
by Anne Morgan Vance

SYMPOSIUM: Research and the Scientific Method in Medical Technology.....363-394

Introduction 363
by Ina Lea Gallaher

Why Research in Medical Technology.....365
by Raphael Cohen

The Scientific Method.....371
by Earle Arnow

Opportunities for Application of the Scientific Method in the Routine Laboratory.....381
by Arthur L. Babson

Problems in Validating Research.....388
by Stephen J. Koziol

Public Health Benefits from Increased Clinical Laboratory Inquiry.....394
by Leonard A. Scheele

ABSTRACTS 350, 369

ANNOUNCEMENT 362

CLASSIFIED ADVERTISING.....XXXIX, XL

CONVENTION 1961.....398

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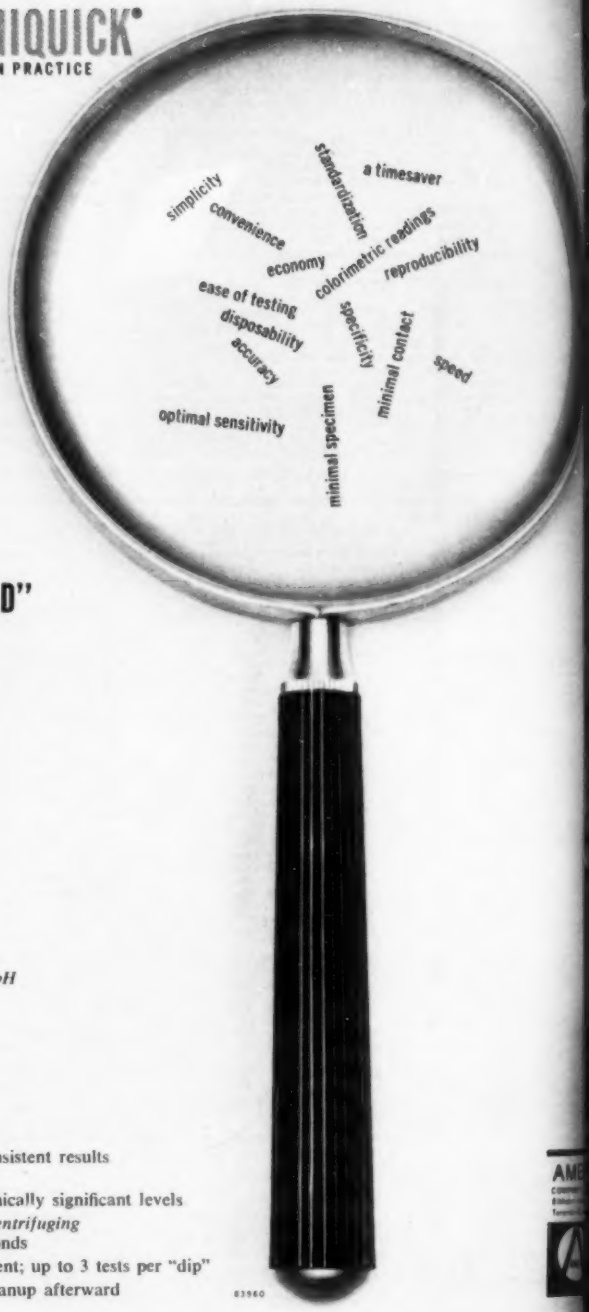

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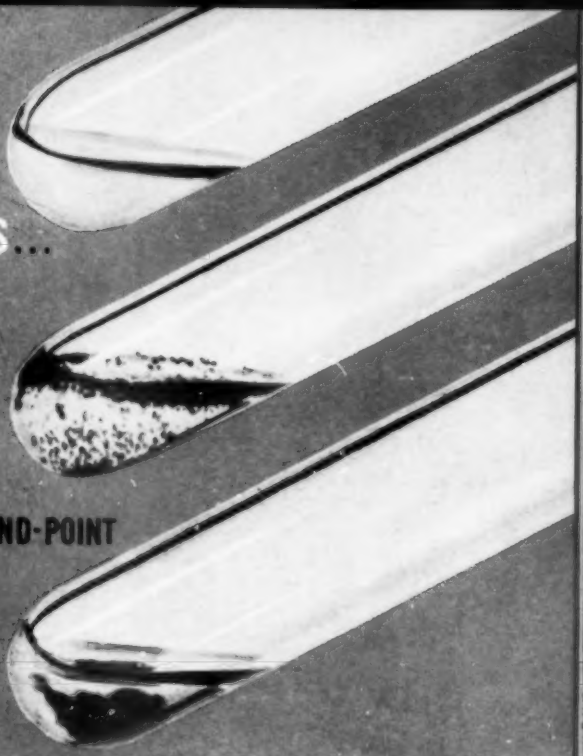

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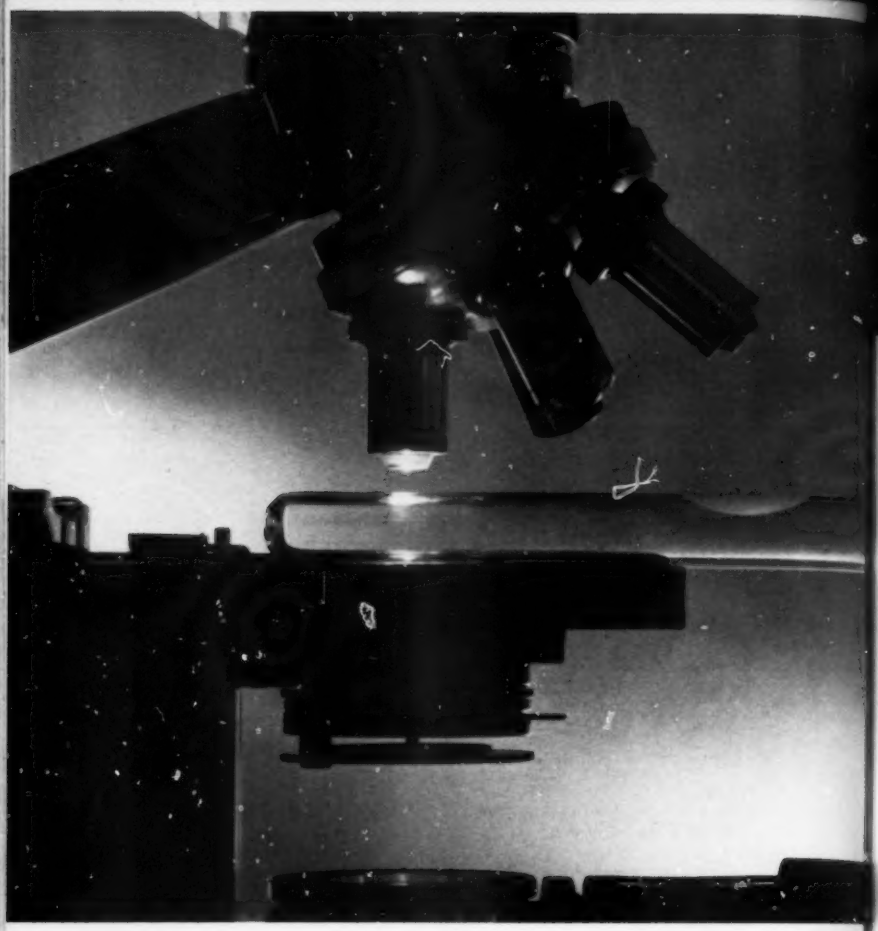

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(1) Ham, T. H.: A Syllabus of Laboratory Examinations in Clinical Diagnosis, Cambridge, Harvard, 1950, p. 249. (2) Lippman, R. W.: Urine and the Urinary Sediment, ed. 2, Springfield, Ill., Thomas, 1957, p. 8. (3) Duncan, G. G.: Diabetes Mellitus, Principles and Treatment, Philadelphia, Saunders, 1951, pp. 65, 66, 78. (4) Williamson, P.: Practical Use of the Office Laboratory and X-ray, St. Louis, Mosby, 1957, p. 41. (5) Todd, J. C.; Sanford, A. H., and Wells, B. B.: Clinical Diagnosis by Laboratory Methods, ed. 12, Philadelphia, Saunders, 1953, p. 114.

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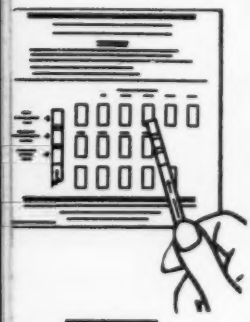

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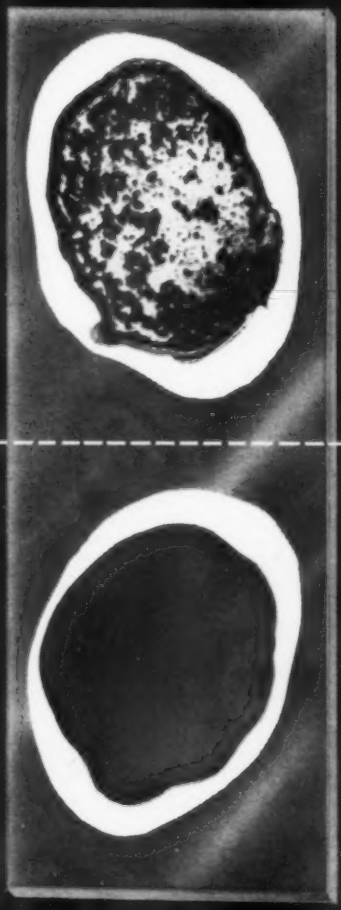

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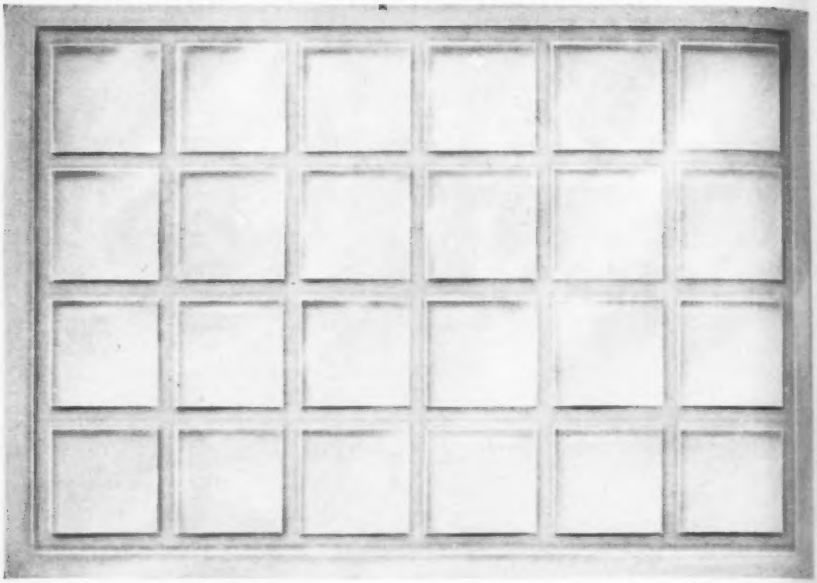

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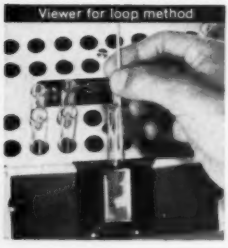
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ABSTRACTS

Babson, A. L., Read, P. A., and Phillips, G. E.: The importance of the substrate in assays of acid phosphatase in serum. Am. J. of Clin. Path. 32:1 83-87, July 1959.

A comparison is made between six generally used substrates for their relative specificity to prostatic and erythrocytic acid phosphatase. Results showed that a new substrate, alpha-naphthyl phosphate, was twice as specific as beta-glycerophosphate and 40-100 times as specific as all other substrates investigated. Acid phosphatase assays in serum should be specific for that enzyme arising from cancerous prostatic tissue. The substrate alpha-naphthyl phosphate* is specific for this enzyme.

Babson, A. L., and Read, P. A.: A new assay for prostatic acid phosphatase in serum. Am. J. Clin. Path. 32:1, 88-91, July 1959.

A new and specific method for the determination of prostatic acid phosphatase in serum is presented. The method is based on the use of a new substrate alpha-naphthyl phosphate which measures only prostatic acid phosphatase. The method is simple and rapid to perform with a minimum of manipulation. In this procedure the color developed represents prostatic enzyme activity alone. The reagents for this new method are available in convenient and stable form.*

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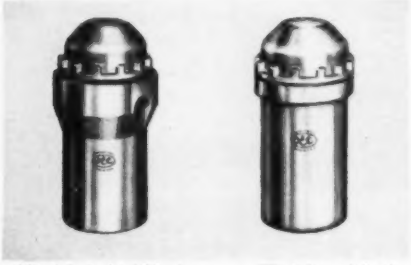
New Laboratory Safeguards Eliminate Danger from Infectious Aerosols

Today, all hospitals and public health laboratories use modern safety measures to protect personnel from the dangers inherent in the infectious materials with which they work.

Despite these safeguards, recent surveys indicate an all too frequently overlooked danger area. Studies show that *airborne* aerosols, resulting from the centrifuging of TB sputum and other infectious materials in unguarded tubes and bottles, are the cause of much of the personnel infection which has been detected.

MAXIMUM SAFETY MEASURES NEEDED

If laboratory work is to be made completely safe for scientist and technician, this "aerosol effect" must be eliminated. Procedures which utilize maximum safeguards will insure protection of personnel.



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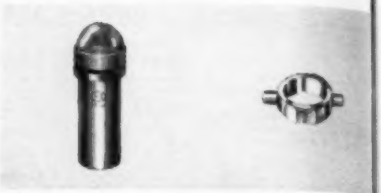
The U. S. Army Biological Warfare Lab., Fort Dietrick, Maryland, found that infectious aerosols had become a serious problem in their germ warfare research. The Army turned the problem over to International Equipment Co. of Boston, Massachusetts, long known for their policy of employing maximum safety measures in all their equipment. After considerable research, International developed sealed accessories for 250 ml cups and bottles. This proved to be the answer to the infectious aerosol problem. These proven accessories are now commercially available, and offer complete protection against the spread of even the most deadly airborne aerosols in the event of tube breakage during centrifuging.



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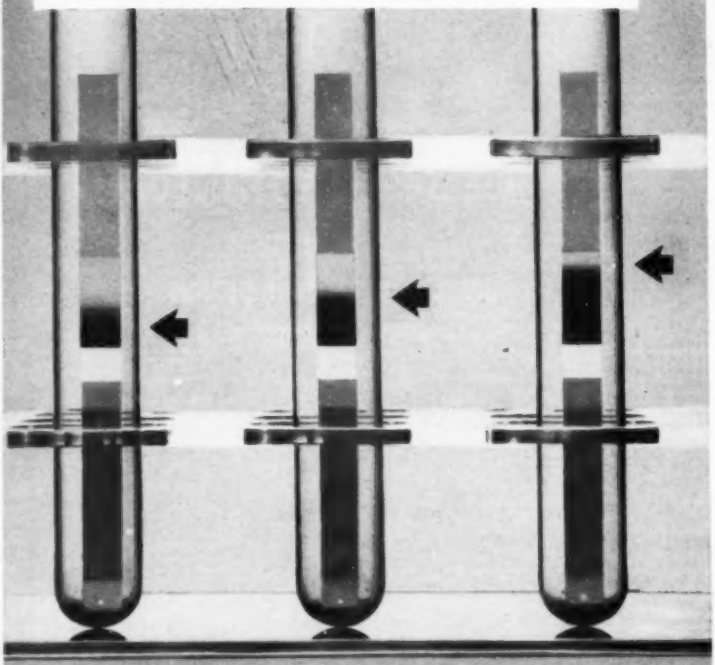

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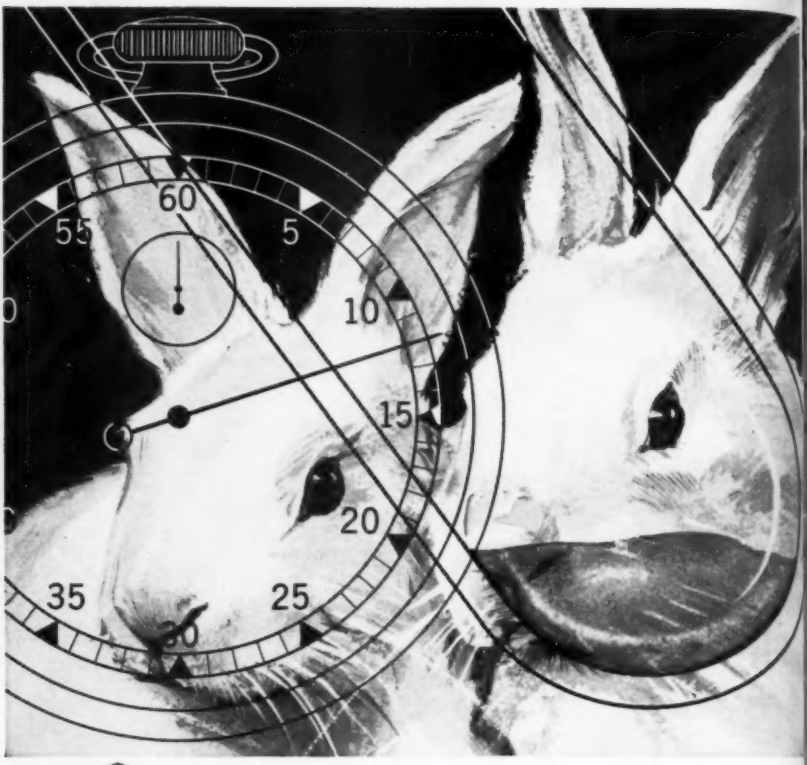

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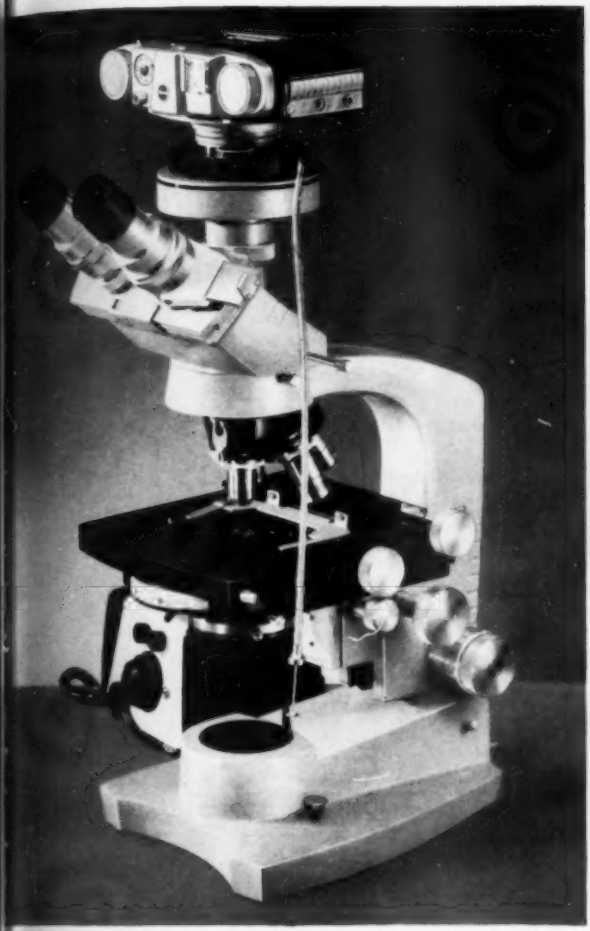

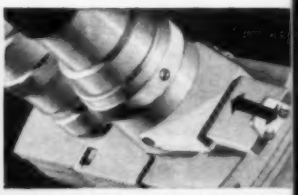

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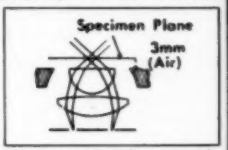

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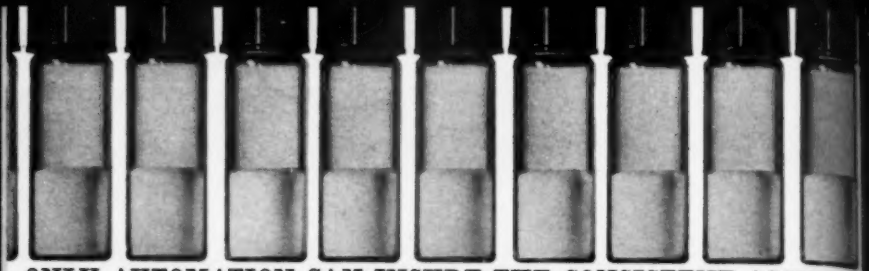

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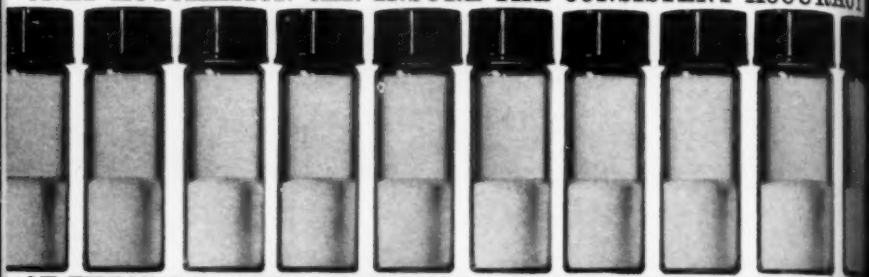

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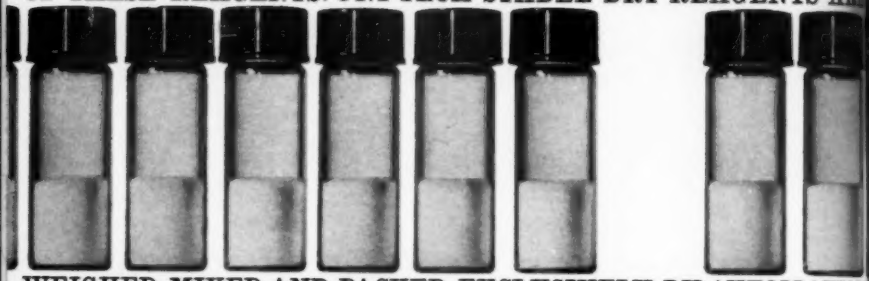

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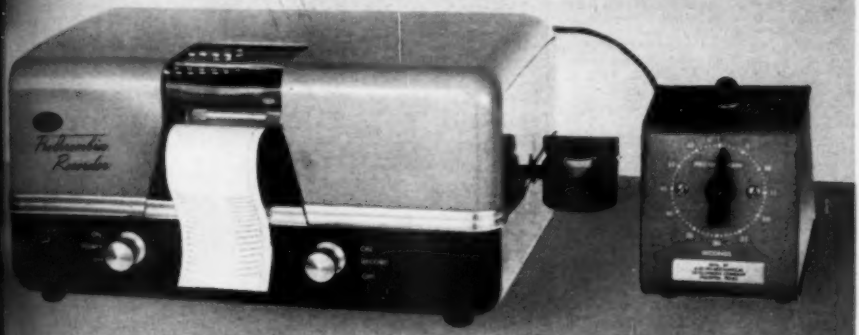

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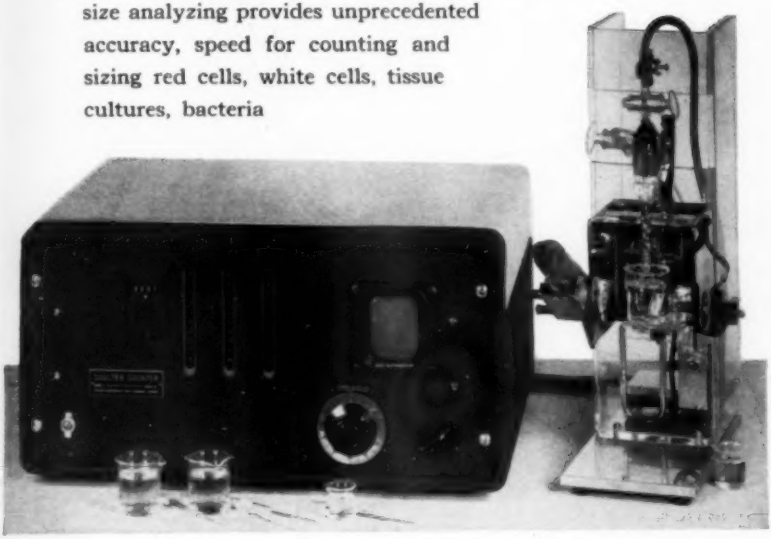

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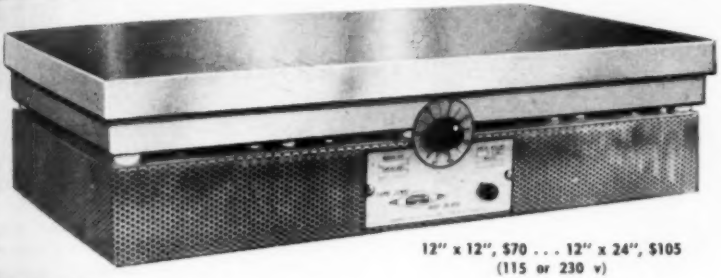

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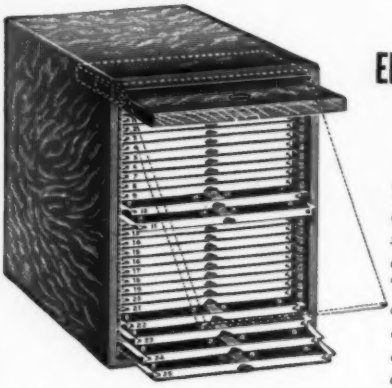

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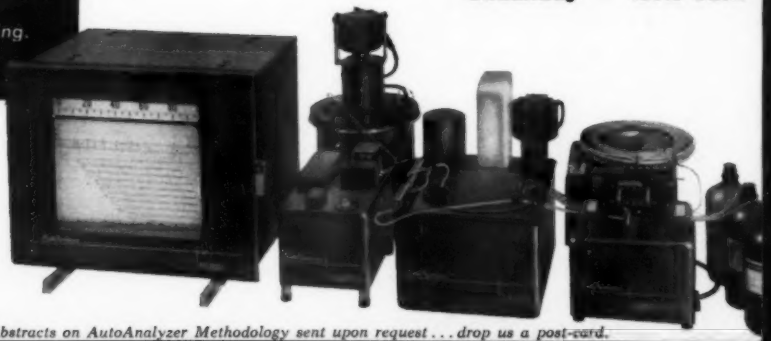

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